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CONTENTS

AFFERENT IMPULSES FROM MUSCULAR RECEPTORS. *G. P. McCouch, A. Forbes and L. H. Rice* 1

THE INFLUENCE OF PROTEIN, BLOOD, LIVER, FAT, IRON AND POTASSIUM IN THE DIET UPON THE RATE OF BLOOD REGENERATION AFTER HEMORRHAGE IN THE RAT AND DOG. *Clive M. McCay* 16

A STUDY OF THE SENSORY AREAS ELICITING THE SWALLOWING REFLEX. *W. T. Pommerenke* 36

CHANGES IN THE SEDIMENTATION RATE OF THE ERYTHROCYTES AND IN THE PLASMA PROTEINS FOLLOWING PROLONGED CHLOROFORM ADMINISTRATION TO THE DOG. *M. D. Rourke and E. D. Plass* 42

THE EFFECT OF PHYSICAL EXERCISE ON THE GASTRIC SECRETION. *Lathan A. Crandall* 48

THE DISTRIBUTION OF ARSENIC IN THE TISSUES AFTER SERIAL ADMINISTRATION OF NEOARSPHENAMINE. *Frank P. Underhill and Alice Dimick* 56

SOME FACTORS INFLUENCING THE NORMAL ISOMETRIC CONTRACTION PERIOD OF THE HUMAN HEART. *Otis M. Cope* 61

THE HEAT LIBERATED BY THE BEATING HEART. SEVENTH COMMUNICATION. A STUDY OF THE SYSTOLIC AND DIASTOLIC TEMPERATURE RISES DURING ANOXYBIOSIS. *Charles D. Snyder* 69

THE INFLUENCE OF YEAST ON THE ALIMENTARY RATE. *D. W. Thorup and A. J. Carlson* 90

THE INFLUENCE OF YEAST ON GASTRIC SECRETION (MAN, DOG). *R. S. Allen and G. E. Burgel* 98

GLUCOLYSIS IN BLOOD SAMPLES. *Anton R. Rose and Fred Schattner* 103

A NEW METHOD FOR THE SIMULTANEOUS DETERMINATION OF MINUTE AMOUNTS OF CARBON DIOXIDE AND OXYGEN. *Wallace O. Fenn* 110

STUDIES OF THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS. XXII. ADRENIN SECRETION ON EXPOSURE TO COLD, TOGETHER WITH A POSSIBLE EXPLANATION OF HIBERNATION. *S. W. Britton* 119

THE THYROID GLAND AND THE SENSITIVITY OF ANIMALS TO INSULIN. *S. W. Britton and W. K. Myers* 132

MEDULLIADRENAL SECRETION AND CARBOHYDRATE METABOLISM. *S. W. Britton, E. M. K. Geiling and H. O. Calvery* 141

THE PREPARATION OF A STERILE, ACTIVE EXTRACT FROM THE ANTERIOR LOBE OF THE HYPOPHYSIS. WITH SOME NOTES OF ITS EFFECTS. *Tracy J. Putnam, Harold M. Teel and Edward B. Benedict* 157

BLOOD AND CEREBRO-SPINAL FLUID CHANGES AFTER INTRAVENOUS INJECTION OF HYPERTONIC SOLUTIONS. *J. M. Kinsman, E. G. Spurling and F. Jelsma* 165

OBSERVATIONS ON PARATHYROIDECTOMIZED DOGS, WITH PARTICULAR ATTENTION TO THE REGIONAL INCIDENCE OF TETANY, AND TO THE BLOOD MINERAL CHANGES IN THIS CONDITION. *C. I. Reed, Robert W. Lackey and J. I. Payle* 176

STUDIES ON THE KNEE JERK. V. THE EFFECT OF HUNGER CONTRACTIONS UPON THE KNEE JERK. *C. A. Johnson and A. J. Carlson* 189

THE EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT. IV. LENGTH OF SEXUAL LIFE. *James Rollin Slonaker* 192

THE INFLUENCE OF YEAST IN THE DIET OF THE RAT UPON THE ACTIVITY OF ISOLATED SEGMENTS OF DUODENUM AND COLON. *Erma A. Smith and Beulah A. Plummer* 200

THE INFLUENCE OF CEREBRAL BLOOD-FLOW ON RESPIRATION. I. THE RESPIRATORY RESPONSES TO CHANGES IN CEREBRAL BLOOD-FLOW. *Carl F. Schmidt* 202

THE INFLUENCE OF CEREBRAL BLOOD-FLOW ON RESPIRATION. II. THE GASEOUS METABOLISM OF THE BRAIN. *Carl F. Schmidt* 223

THE INFLUENCE OF CEREBRAL BLOOD-FLOW ON RESPIRATION. III. THE INTERPLAY OF FACTORS CONCERNED IN THE REGULATION OF RESPIRATION. *Carl F. Schmidt* 242

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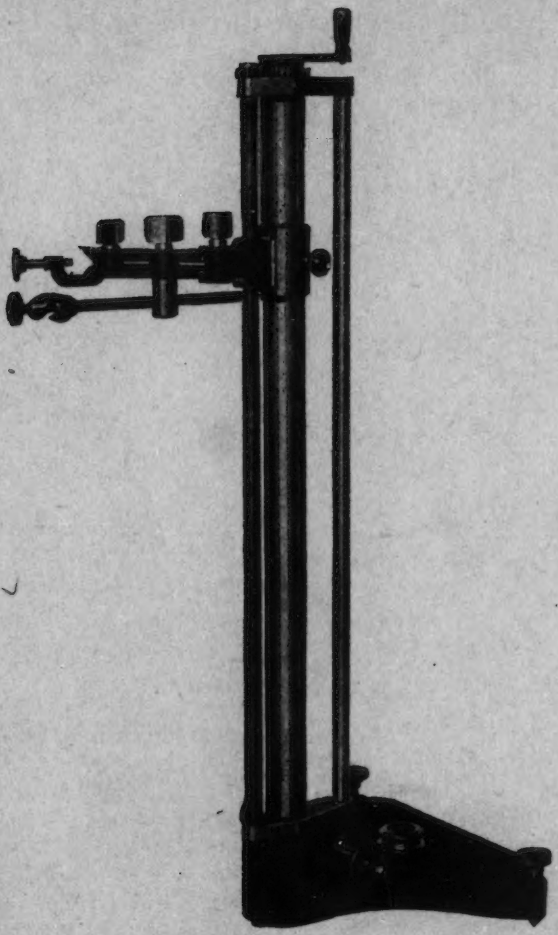

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AFFERENT IMPULSES FROM MUSCULAR RECEPTORS

G. P. McCOUCH, A. FORBES AND L. H. RICE

From the Laboratories of Physiology, Harvard, and Department of Physiology, University of Pennsylvania

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The study of the afferent impulses from muscles should yield useful data for the analysis of proprioceptive reflexes. Forbes, Campbell and Williams (1) have described a series of waves obtained with the string galvanometer from the peroneal nerve of the cat following the neuromyal twitch from a single induction shock. The nerve was dissected from the popliteal and cut at the level of the hip. To obtain monophasic records, Sherrington stimulating electrodes were placed distal to the moist chamber containing the leads between which the nerve was crushed. The arrangement is shown in figure 1 adapted from their paper.

Records were obtained both with movement of the foot unrestricted, and with movement cut short by a string connecting the foot to a fixed point. These show four waves: *a*, the action current of the motor impulses set up by the stimulus; *b*, *c* and *d* three small waves due to groups of afferent impulses. Figure 2 (taken from their paper) is a diagram of such an electroneurogram. The proprioceptive origin of *b*, *c* and *d* was indicated by their temporary disappearance when an alcohol block was introduced between stimulating electrodes and muscle. With increasing limitation of movement, *c* approaches and merges with *b*, while *d* decreases and disappears. An analysis of the time relations inclined the authors to the view that all three groups of proprioceptive impulses were responses to rise of tension. The present work was undertaken in the hope of obtaining further data for the test of this hypothesis.

METHOD. The experiment was repeated upon ten decerebrate cats with the following modifications in technique: 1. Crushing the nerve at the end of the experiment was used as a control instead of the alcohol block. 2. Records of action currents, of tension, and of movement were obtained synchronously upon the same film. 3. Action currents were recorded with a relatively slack string (256 meters per ampere) without amplification.

4. Tension was recorded by a small torsion lever sewed into the tendon of the tibialis anticus muscle, the tendon being cut between the points of attachment to the lever.[1] Upon each arm of the lever was mounted a flat wound coil of fine copper wire, the pair forming an inductorium. The primary coil was connected in the plate circuit of a vacuum tube oscillator of higher frequency than the galvanometer could follow. The secondary coil connected the grid and negative pole of the filament of a rectifying tube. Arranged in the plate circuit of the rectifying tube was the string of a Salamonson galvanometer (see fig. 3). The lever was calibrated for tension by weights. Its latency was determined by connecting it in series with a lever of known latency and a falling weight and found to be 10σ.

To record movement, a string tied to the foot extended horizontally

S, secondary coil of stimulating inductorium; *G*, galvanometer; *N*, nerve; *M*, muscle; *LL*, leading-off electrodes; *B*, cold block; *T*, thermometer.

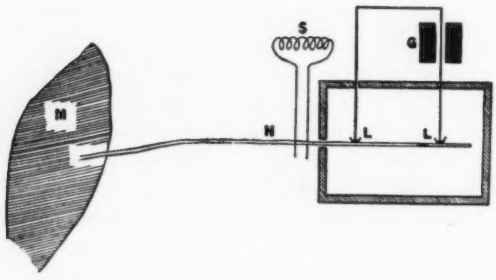

Fig. 1

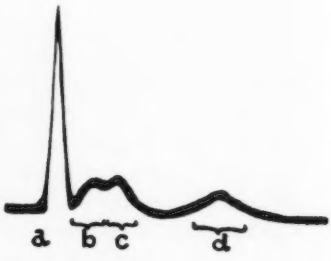

Fig. 2

Fig. 1. Diagram of arrangement of apparatus for recording action currents adapted from Forbes, Campbell and Williams.

Fig. 2. Diagram of electroneurogram taken from Forbes, Campbell and Williams.

below the camera slit to a light spring, the end of which was attached to a rigid support. A fine silk thread tied to the string was stretched vertically in front of the slit to a fixed point about 3 feet above the camera. The latency of this system is appreciable in movement toward the spring, negligible in movement away from it. This was determined by a comparison between two sets of records of movement and proprioceptive action currents. In one, the stimulus was applied to the peroneal nerve and the galvanometer leads to the popliteal nerve. In the other, the popliteal was stimulated and the action current of the peroneal recorded. In the first series, the galvanometer deflection began before that of the movement record; in the second series, after it (fig. 6, A, B and C). In flexion of the foot the movement record begins after a latency of 6.4σ; but

[1] This modification of a method described by one of us (2) will be dealt with more fully in a future communication.

the peak of the curve denotes the peak of movement without appreciable latency.[2]

An attempt to correlate time relations is complicated by the fact that the waves of the action current record are not isolated. The galvanometer string remains above its base line from the beginning of the *a* wave until the end of the *d* wave. The beginning of each wave is buried in the descending limb of its predecessor. Consequently only peak measurements have been utilized in the analysis of time relations. Even the peak of *b* may be hidden. Only the *a* wave represents the response to a single volley of synchronous impulses. *c* and *d* are dome-shaped waves of such gradual slope that even the determination of the peak may have an error of 1 to 2σ. Comparison of the peak of movement with the peak of the *c* wave may give a combined error of 4 to 5σ.

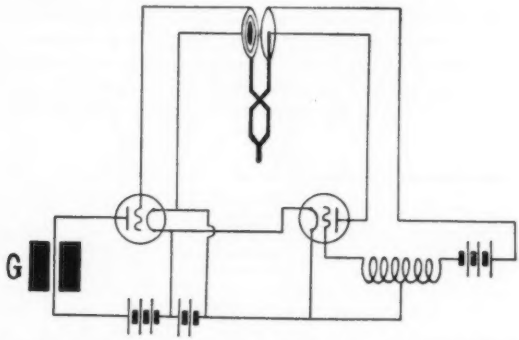

Fig. 3. Diagram of arrangement of apparatus for recording tension.

A more serious difficulty arises from the high threshold (about 100 grams and considerable inertia of the tension lever. The curve which it records has no readable notch corresponding to the dip in the action current curve between *b* and *c*. But it is doubtful whether it would follow fluctuations of this size and rate.

RESULTS. The more significant results are summarized in tables 1 and 2. From table 1 it is evident that where movement is unrestricted the peak of the *b* wave slightly precedes the peak of tension, falling at the time when tension is increasing most rapidly. The *c* wave bears a similar relation to movement but does not show an obvious relation in time to the curve of tension. When movement is limited, however, the peak of the tension record falls far behind the peak of *b*, very near to that of *c*, and approaches the peak of movement to an extent corresponding to the degree of limitation.

[2] Dr. J. F. Fulton has kindly called our attention to the failure of this estimate to allow for overshooting. Small allowance for this factor would strengthen the evidence presented for our conclusions concerning the *c* wave.

Table 2 correlates the degree of tension and of movement with the intensity of the proprioceptive response. The highest values of the *bc* complex occur neither with maximal tension nor maximal movement, but with moderate limitation where both tension and movement attain moderately high values.

TABLE 1

Time intervals between peaks in σ

NUMBER OF RECORDS	NUMBER OF EXPERIMENTS	DEGREE OF LIMITATION	*b* TO TENSION	TENSION TO *c*	*c* TO MOVEMENT*	TENSION TO MOVEMENT†
28	6	None	2.4	14.5	8.0	23.1
10	4	Slight	9.1	12.9	7.8	15.4
12	5	Moderate	14.7	6.0	3.5	9.1
11	7	Great	17.8	0.6	5.1	7.0
6	4	Extreme	14.8	2.0	4.4	6.4

* No allowance has been made for overshooting of the movement record.

† The lack of agreement of the sum of columns 5 and 6 with column 7 is due to the inclusion in the calculation of column 5 of several records not included in that of column 6 because the peak of the movement record was off the film.

TABLE 2

LIMITATION	RESISTANCE IN PRIMARY CIRCUIT (INVERSELY PROPORTIONAL TO STIMULUS)	HEIGHT		TENSION	MOVEMENT
		a	*bc*		
	ohms	*mm.*	*mm.*	*grams*	
Extreme	600	4.6	0.6	800+	1.2
Great	500	5.4	1.1	800	1.7
Moderate	500	5.7	1.2	544	1.5
Moderate	600	4.2	1.3	352	2.3
Slight	600	4.8	1.2	290	2.3
None	600	4.8	1.0	243	3.8
None	500	5.7	0.8	237	4.0
None	500	5.8	0.8	212	4.0
None—Popliteal n. cut	500	6.1	0.6	220	4.1
Extreme	200	6.6	1.7	590	0.3
Extreme	200	6.2	1.4	760	0.3
Moderate	200	6.2	1.9	635	2.1
None	300	4.6	1.0	450	4.1
None	200	6.1	1.5	515	4.8
None	200	6.3	1.55	435	5.7
None	100	7.7	2.8	460	6.15
None—Popliteal n. cut	200	6.5	1.6	255	6.1
None—Popliteal n. cut	200	6.6	1.5	260	6.3
None—Achilles ten. cut	200	6.7	1.6	100	4.6

Data from experiment of March 1st above.

Data from experiment of March 2nd below.

ANALYSIS OF RESULTS. The time relations obtaining between the peaks of tension, of movement, of the *b* wave, and of the *c* wave vary somewhat from experiment to experiment, but show certain consistent variations with different degrees of limitation in all experiments (see figs. 4 and 5). A perfect record of tension where movement is limited would have two peaks: the first due to initial tensile rise at the onset of contraction, followed by a fall as the muscle shortens and the foot gains momentum; the second due to the sudden check to shortening giving an isometric phase to the remainder of contraction. The relative heights of the two peaks would vary with the degree of limitation, the greater the limitation the higher the second peak. When limitation occurred within the period of initial tensile rise, there would be fusion into a single peak. The majority of our tension records failed to show the two peaks. When tension is increased by limitation, the peak of the record represents the second peak. It is probable

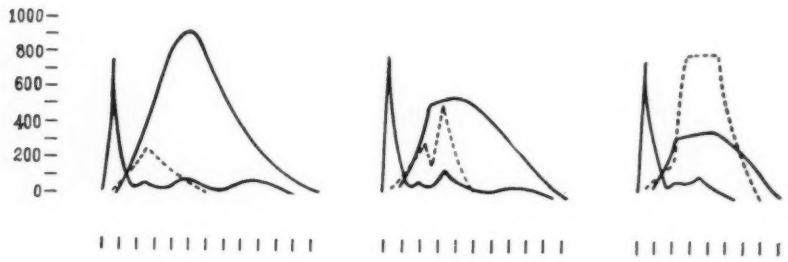

Fig. 4. Diagram of records of action currents, tension and movement superposed upon the same base line. Dotted line denotes tension and may be read in grams by the scale upon the ordinate. Time in 0.01 second. The degree of limitation may be measured by the height of the movement record.

that friction from tissues with which the lever is in contact, is the chief factor in this distortion. Such friction introduces an undetermined variable not accounted for in our determinations of latency. To this we attribute most of the variation between experiments in the position of the peak of tension. It is noteworthy that the only experiment in which the tension record shows a double peak consistent in time and height with the degree of limitation is the only one in which, in records without limitation, the peak of the *b* wave falls after the peak of tension. In this experiment, (February 25th) in records without limitation, the average interval from the peak of tension to the peak of *b* is 3.1 σ; the average position of the peak of *c* is 21.7 σ after the peak of tension and 5.2 σ before the peak of movement. It is significant that the curve of movement begins abruptly but slows progressively during the 10 σ preceding its peak. These time

relations taken together suggest that when movement is not limited, the *b* wave consists chiefly of impulses from receptors stimulated by rise of tension, the *c* wave chiefly of impulses from receptors responding to move-

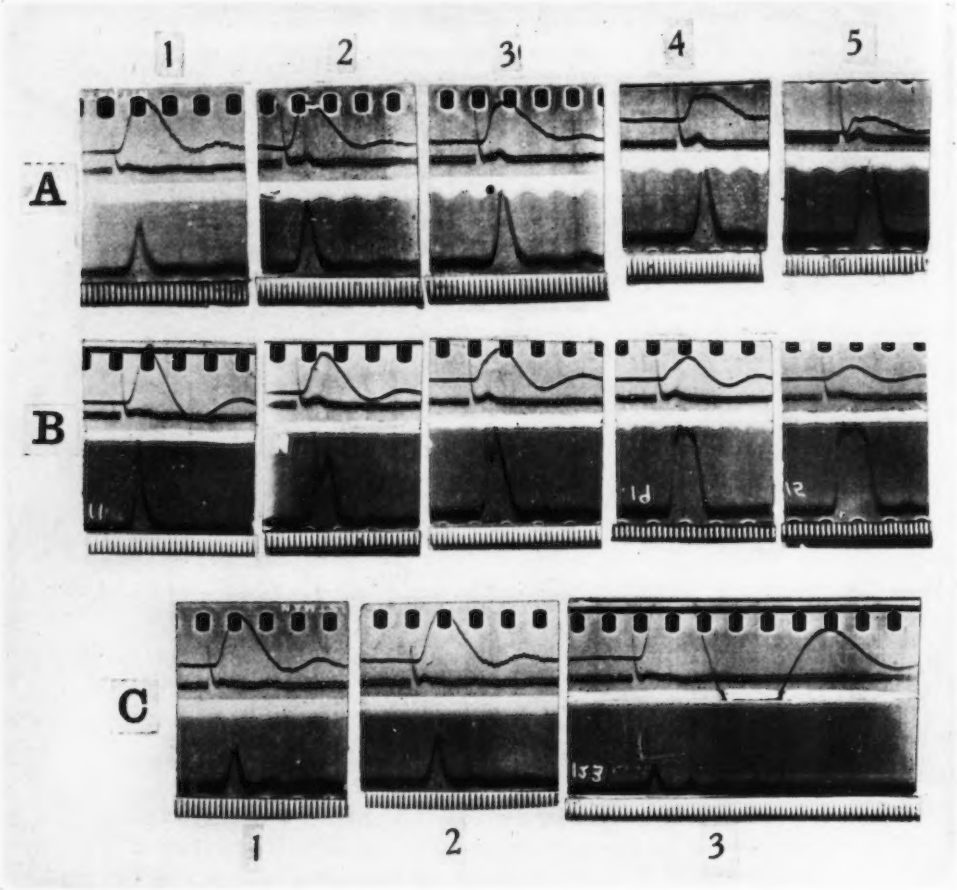

Fig. 5. Leads arranged as in figure 1. Top line records movement. Middle line records action currents. Bottom line records tension. Below time, 0.01 second per double vibration.

A, experiment of February 18th. B, experiment of February 25th. In A and B; *1*, no limitation of movement; *2* to *5*, progressive increase of limitation. C, *1* and *2* from experiment of February 18th. C, *3* from experiment of February 25th. C, *1* no limitation, popliteal nerve intact. C, *2* no limitation, popliteal nerve cut. C, *3* no limitation, Achilles tendon cut. Note the presence of the *c* wave in all 3 records.

ment. Since the curves of tension and movement overlap, it is probable that the peak of the *b* wave is delayed slightly by components due to movement, and the peak of the *c* wave is advanced by components due to tension. It may be significant that the experiment in which the proprioceptive peaks are earliest, *b* averaging 5.6 σ before the peak of tension and *c* 10.7σ before that of movement, is that which affords least evidence of proprioceptive response to movement; and the experiment in which the proprioceptive peaks are latest, *b* averaging 3.1 σ after the peak of tension, and *c* 5.2 σ before that of movement, is one in which movement appeared to be an important source of stimulation. Such a coincidence favors the view that the peak of *b* is retarded by a component due to movement to a position later than it would have attained from the component due to tension alone, and that the peak of *c* is advanced by a component due to tension to a position earlier than that determined solely by movement. The data are summarised in table 1.

In the case of the *c* wave another possiblity must be considered. The flexion of the ankle stretches the gastrocnemius and soleus which may respond with a myotatic reflex, thus putting sudden tension upon the flexors. Such a secondary tensile rise may indeed contribute to the *c* wave. That it does not contribute a major component is proved by the persistence of the *c* wave after section of the popliteal nerve and even of the Achilles tendon (see fig. 5, *c*).

In considering the origin of the *c* wave, a distinction must be made between the records without limitation and those with limitation. In the former the *c* wave is dome shaped, in the latter it is a sharp peak similar in shape to the *b* wave. Such records are shown in figure 5, A and B, and illustrated diagrammatically in figure 4. Where the tension record shows two peaks, the *c* wave bears a relation to the second peak similar to that of the *b* wave to the first. In a record in which the *b* wave precedes the first tensile peak by 8 σ, the *c* wave precedes the second by 10.5 σ; and in another record the latter interval is 5.4 σ. In experiments in which the tension record shows but a single peak, this falls farther behind the *b* wave and closer to the *c* wave as limitation increases until with extreme limitation it is almost synchronous with the latter. These relationships suggest that with increasing limitation, tension contributes to the *c* wave an increasing number of impulses, which probably become the major component with even moderate limitation.

A prominent feature of both *b* and *c* is the early position of the peak before the peaks of tension and of movement respectively. In records without limitation, the peak of *b* precedes that of tension by an average of 2.4 σ, and the peak of *c* that of movement by an average of 8 σ. This precedence cannot be attributed entirely to artefacts in recording of tension, for it is present to a marked degree in records of the response to passive stretch

from the twitch of antagonistic muscles. This was evoked by stimulating the peroneal nerve and leading off from the popliteal. In this experiment the peak of tension must coincide with the peak of movement, which is recorded without appreciable lag (fig. 6, B and C). The proprioceptive peaks seem related less to the absolute maxima of stimulation than to their gradients, the times when they are increasing at a maximum rate.

In the experiments so far considered, joint movement, tension upon tendon and muscle, and change of muscle shape may all contribute to the proprioceptive complex. In two experiments the first two factors were eliminated by section of all tendons and the results controlled by repetition after crushing the nerve. Records from these are shown in the lower four rows of figure 6. In both cases it is possible to pick out upon the film, the point at which the crush occurred from a gross comparison of the shape or duration of the descending limb of the *a* wave. In only one of these does measurement indicate a possibly significant decrease in duration after crushing, and this averages only 3.3 σ (table 3). Under these conditions, however, there are two factors which may obscure a positive result. In

TABLE 3

Tendons cut May 26th. Duration of A wave in σ

	NUMBER OF RECORDS MEASURED	PEAK TO END			START TO END		
		Maximum	Minimum	Average	Maximum	Minimum	Average
Before crushing nerve.....	18	23.2	16.2	18.8	25.5	18.6	21.6
After crushing nerve.....	16	18	13.3	15.5	20.9	16.8	19.4

the first place, the mere release of a tendon from its insertion induces a degree of shortening beyond the normal range, thus limiting the further change in length upon stimulation. In the second place, when unrestrained by the inertia of the foot, the change in length will occur so early that any afferent impulses it may induce might be obscured in the galvanometer record by the descending limb of the *a* wave, as frequently occurs in the case of the *b* wave when the tendons are intact. On the other hand, the smooth course of the descending limb argues against the presence of such a smothered proprioceptive component. An unobserved shift of the nerve upon the leads when crushing would account for the slight differences observed (3). The result is inconclusive; yet it supports the view that a response associated with movement and independent of tension upon the tendon arises in muscular receptors. The early position and small magnitude of this response suggest that it is associated with contraction and confined to the *b* wave and the early part of the *c* wave. Were the response from intramuscular receptors subtracted from the total proprioceptive complex, the remainder of the *c* wave would probably attain its peak at the point of extreme flexion of the ankle joint. It is probable that this posture

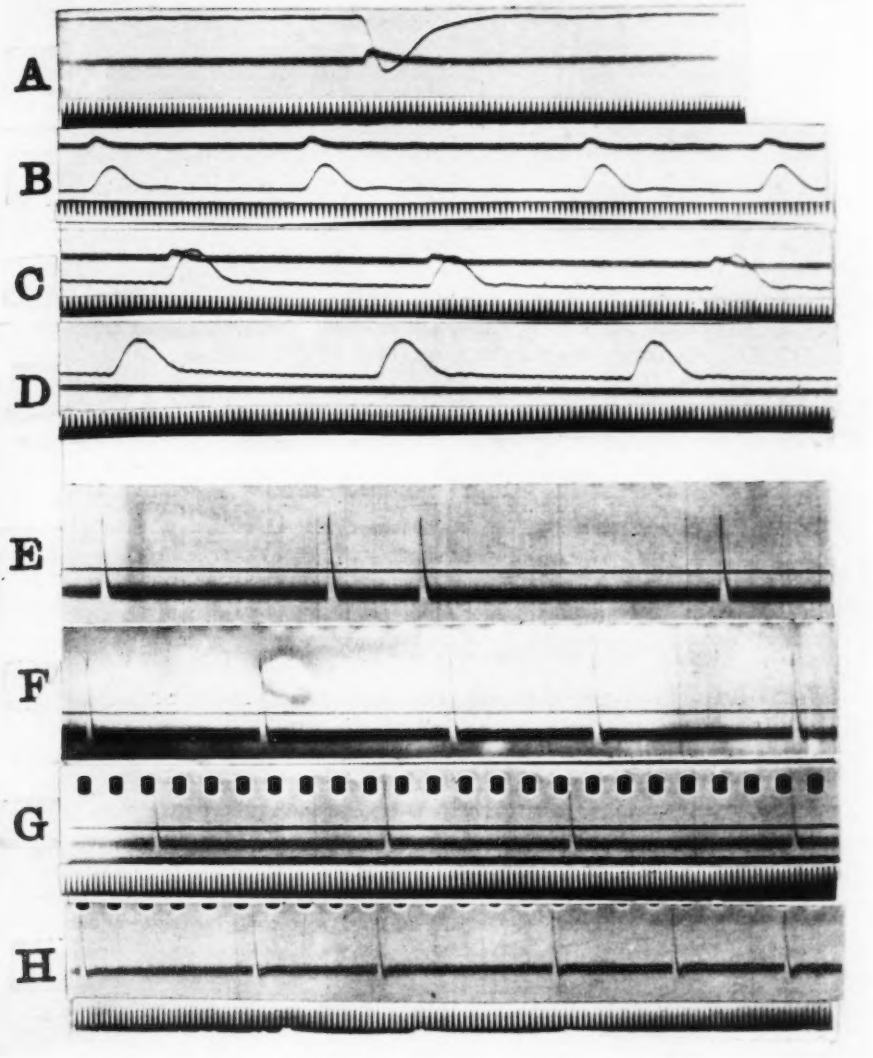

Fig. 6. Records A to D of movement and action current from experiment of March 1st.

A, stimulus to popliteal galvanometer leads from peroneal. B, stimulus to peroneal galvanometer leads from popliteal (knee flexed). C, stimulus to peroneal galvanometer leads from popliteal (knee extended). D, stimulus to peroneal galvanometer leads from popliteal (popliteal crushed). Records E and F from experiment of May 26th after section of all tendons. E before crushing the nerve. F after crushing the nerve. Records G and H from experiment of February 26th after section of all tendons. G, before crushing the nerve. H, after crushing the nerve.

stimulates chiefly the anterior portion of the joint, which is the part supplied by the peroneal nerve, from which our records were obtained. Such considerations suggest an articular origin of a considerable part of the *c* wave.

Whatever the location of the receptors, the existence of proprioceptive impulses due to movement is indicated, not only by the time relations, but also by a comparison of the degree of tension and of movement with the size of the proprioceptive response. For this purpose, peak values were employed. These were measured by a microscope with crossbars in the eyepiece and were read by the vernier of the mechanical stage holding the film. In a few cases the film was projected by lantern upon paper and the total area of the *bc* complex compared with that of the tension curve. As anticipated, this method gave relatively higher values for proprioceptive action currents in records with little or no limitation of movement, owing to the greater separation of *b* and *c* waves. But the difference was not great enough to warrant the routine use of a method which is not only more tedious, but less accurate than readings of height obtained with a microscope. Had the computation of total amount been employed, it would have strengthed the evidence for a component due to movement. For the summation due to overlap of the responses to tension and to movement increases the height of the peak and the overlap is proportional to the degree of limitation.

The important work of Adrian (4) and of Adrian and Zotterman (5) on the response of muscle receptors to stretch, has proved that even when the number of receptors involved is constant, extensive gradation may occur through increase in frequency of response to increase in intensity of stimulation; that such increase in frequency is not sufficient to encroach upon the relative refractory period of the nerve fibre and hence involves no reduction in amplitude of the axone action potential; and that a curve of frequency of response plotted against intensity of stimulus follows a smooth course. In our experiments, such an increase in frequency would increase the percentage of fibres in active phase under the galvanometer lead at a given instant. This may be the only type of gradation occurring when maximal induction shocks are employed and in this case the only variables would be those incident to change in the degree of limitation of movement. The stimuli we employed were only approximately maximal. Fortunately, we have an index of the proportion of fibres contracting in the height of the *a* wave which is the immediate response to the stimulus. Were tension the only adequate stimulus, we should expect a plot of height of the *bc* complex divided by the height of *a* (percentage of fibres contracting) as ordinate against tension as abscissa to approximate a smooth curve not passing through a maximum, provided no cases be included in which the number of fibres responding is so small as to increase markedly

the load per fibre in records without limitation. Such a graph actually presents a disorderly scattering of points, but with a tendency to pass through a maximum. A similarly scattered series results from plotting the same ordinate against movement as abscissa. On the other hand, when

$$\frac{\text{height of } bc}{\text{height of } a \times \text{tension}}$$

is plotted against movement, or

$$\frac{\text{height of } bc}{\text{height of } a \times \text{movement}}$$

is plotted against tension, in order to bring out the components due to movement and tension respectively, smooth curves are approximated as closely as might be anticipated in view of the high percentage error in measurement of values as small as the height of the *bc* complex. These graphs are shown in figure 7. The character of curve varies from experiment to experiment with the relative prominence of the *b* and *c* wave and with the degree to which they overlap each other. Thus in the experiment of March 1st the *c* wave is well developed and clearly separated from *b*, whose peak is often hidden in the descending limb of *a*. Here the curves are concave to the abscissa indicating the high values obtained with moderate limitation when both tension and movement are moderate in degree and the overlap is great. In the experiment of March 2nd, there is no trough between *b* and *c* even in records without limitation.[3] Here the factor of separation of the waves is negligible. Hence, the curves are convex to the abscissa, indicating the dominance of the response to tension when movement is restricted and of the response to movement when it is unrestricted.

For a quantitative estimate of the contribution to the proprioceptive response of each type of stimulation, our data are inadequate. For the conclusion that movement initiates a considerable quota, perhaps the strongest evidence is the fact that in most experiments, the highest values of *bc* are found not in records with extreme limitation in which tension is maximal, but in those with intermediate degrees of limitation in which both tension and movement attain moderate values. This result is seen in figure 5, B and in table 2.

Time relations and variations in magnitude of tension and movement

[3] The high threshold for stimulation in this experiment suggests that the absence of distinct peaks in the proprioceptive complex may be due to slow conduction in fibres injured in dissection of the nerve.

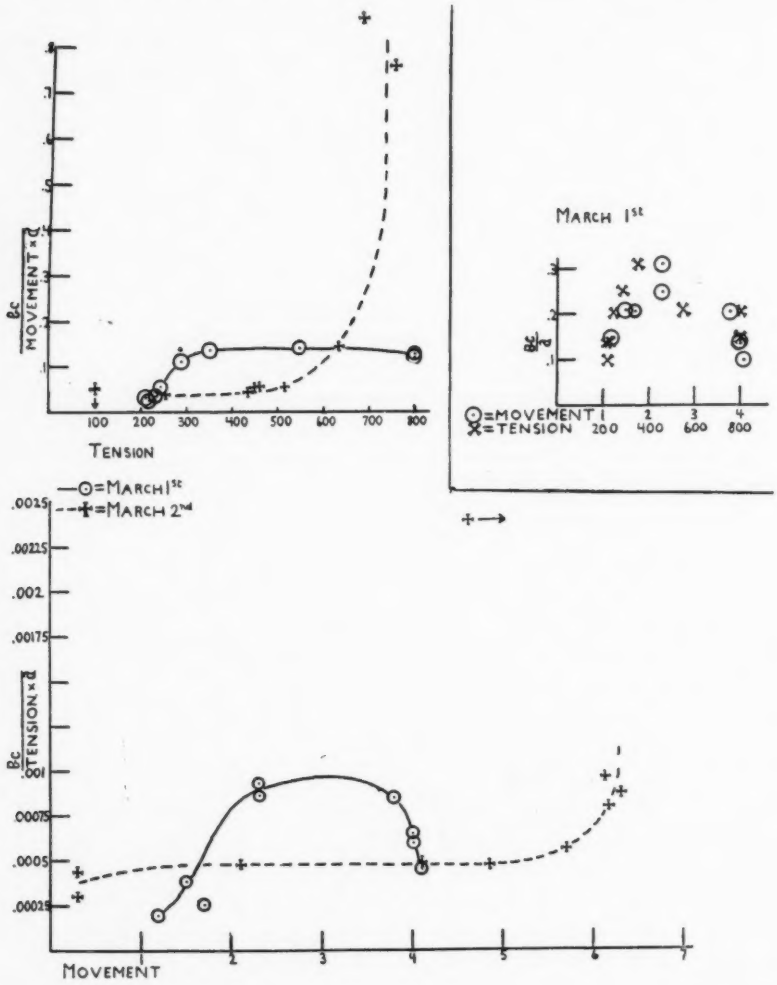

Fig. 7. Points marked with an arrow were calculated from a record obtained after cutting the Achilles tendon. Here the resting position was with the foot considerably flexed, thus limiting the available range of movement. The arrow indicates the direction in which the point would be located by plotting against degree of flexion instead of movement.

throw little light upon the origin of the *d* wave. This wave is prominent in records with no limitation of movement, absent in those with close limitation. It is long and dome-shaped like the *c* wave. It begins when the foot has fallen about half way to the resting position, when the rate of movement begins to decrease. It may end at any point within the 15σ preceding the end of movement. Such a relation suggests the possibility of a rise in tension from the momentum of the falling foot putting the flexors on a stretch. But the tension record fails to support this view. The tension developed in the tibialis anticus during the *d* wave is below the 100-gram threshold of the tension lever. It is true that secondary waves occasionally occur in the tension record, but these are due to vibration of the coils on the lever arms and not to tensile change. They frequently occur in records with close limitation of movement in which there is no *d* wave, and when they occur in the presence of a *d* wave, they are obviously dissociated from it in time. On the other hand, tensions well above the threshold of the tension lever, when elicited by submaximal stimuli, may fail to induce a *b* wave comparable in height to the *d* wave following a maximal contraction. Yet it must be remembered that in such submaximal contractions, tension is developed in only a small percentage of fibres, whereas the passive stretch may involve the entire muscle. Though the passive stretch falls upon a greater number of units and therefore might yield a greater proprioceptive response if the stimulus were effectual, the tension per unit is correspondingly diminished and with it the probability of attaining the thresholds of the tension receptors.

Faced with such negative evidence in regard to tension, let us consider the possibility of movement as an adequate stimulus for the *d* wave. Here there is an obvious difficulty. The phase of relaxation is associated with the decline of the *c* wave. The period of most rapid lengthening of the flexors is marked on the action current record by the trough between the decline of the *c* wave and the rise of the *d* wave, i.e., by a smaller number of afferent impulses than at any other point on the *bcd* complex. It is with the decline in rate of lengthening, with the phase during which the muscle is stretched by the falling foot, that the *d* wave develops. If it be a response to lengthening of the flexor muscles, the threshold for its receptors is attained only when the foot is extended beyond the semiflexed posture. Their response to greater extension may be seen in the records wherein the change of length occurs in the range employed in isometric recording,—those in which the stimulus is stretch from the twitch contraction of the antagonists (see fig. 6, A, B, C and D). Here the proprioceptive response occurs early and abruptly with the peak at the period of most rapid elongation. The action current begins from 2.4 to 6σ after the onset of stretch and attains its peak about 25σ before the peak of stretch. Unfortunately these records fail to distinguish between tension, muscle lengthen-

ing, and joint movement as sources of stimulation. But in the records of the *bcd* complex the decline of the action current between *c* and *d* argues strongly against an articular origin of the latter if it be the chief factor in the former.

DISCUSSION. Our results do not afford sufficient evidence to assign the components of the proprioceptive complex to specific receptors. Still less can we apportion their influence upon sensation and reflex action. Yet it appears significant that they accord with the view advanced upon other grounds by Pi Suñer and Fulton (6) which attributes the stretch reflex to muscle spindles which are regarded as recorders of lengthening. Their electromyograms of the knee jerk show a cessation of action currents in muscle during the period of contraction, suggesting a stoppage of stimulation. Action currents recur abruptly at about half relaxation, at the time of onset of the hump or myotatic appendage to the reflex contraction. It appears significant that this is about the time of the *d* wave in our records. Moreover, the *d* wave in our records, the myotatic appendage and the action current associated therewith in their records of the patellar jerk all appear proportional to the degree of lengthening. Furthermore, Asayama has demonstrated the stretch reflex in the tibialis anticus muscle and shown that the tendon and immediately adjacent end of the muscle are not essential for its production (8). In the light of their work, the major components of the proprioceptive complex in records without limitation might be attributed to the following sources:

b chiefly to Golgi end organs (tension); a small component to muscle receptors (shortening)
c to muscle and joint receptors (movement)
d to muscle spindles (lengthening)

The early peak and rapid decline in the waves of proprioceptive origin recalls an apparently similar phenomenon recorded by Adrian and Zotterman (5) and attributed by them to a fall in excitability of the receptors. Two considerations render it improbable that this is a major factor in our experiments. The first is the early decline of the proprioceptive wave about 12σ after its onset (fig. 6, A, B and C). The second is the greatly enhanced response immediately thereafter, evinced by the sharp early *c* wave with partial limitation of movement (figs. 4 and 5). If we are right in attributing this change in *c* wave to a component due to tension, such an increase in response argues against a high degree of adaptation or fatigue in the tension receptors. Our results may be more readily explained upon the assumption that a large number of receptors stimulated at approximately the same moment are responding at different frequencies. The progressive decrease in the number of responses in phase with each other might reduce the excursion of the galvanometer string. Yet it seems

improbable that such progressive asynchronism accounts completely for the close correspondence between the peaks of the string excursions and the maximum rate of increase in intensity of stimulation. The *b* wave passes its peak as the slope of the tension curve becomes less abrupt, and the *c* wave bears a similar relation to the curve of movement. The same feature is evident in the responses to stretch. Whatever the explanation, the dependence of degree of response upon the stimulation gradient may be related to certain features of the reflex response to abrupt passive movement. Thus Liddell and Sherrington (7) observed that when the myotatic reflex is induced abruptly, it attains an initial peak of tension slightly above the plateau level maintained throughout the period of stimulation. The umklämmerungsreflex of infants described by Moro (9) and further analyzed by Schaltenbrand (10) may depend upon the same factor.

SUMMARY

A comparison of the groups of proprioceptive impulses in the peroneal nerve of the cat described by Forbes, Campbell and Williams (1) with synchronous records of tension of tibialis anticus muscle and of movement of the foot lead to the following conclusions:

1. It is probable that none of the waves represent afferent impulses arising from a single type of receptor.
2. The major component of the *b* wave results from rise of tension.
3. The *c* wave appears to result chiefly from movement.
4. The origin of the *d* wave is uncertain.
5. The greatest proprioceptive responses occur in records with moderate limitation of flexion in which both tension and movement attain moderately high values.

It is a pleasure to acknowledge our indebtedness to Mr. William Forbes for a suggestion which remedied a serious defect in the method of recording tension, and for his skillful mounting of two galvanometer strings; and to Dr. John F. Fulton for helpful criticism in revision of the paper.

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THE INFLUENCE OF PROTEIN, BLOOD, LIVER, FAT, IRON AND POTASSIUM IN THE DIET UPON THE RATE OF BLOOD REGENERATION AFTER HEMORRHAGE IN THE RAT AND DOG

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The experiments reported in this paper were undertaken to determine whether an organic factor as well as iron is of major importance in the regeneration of hemoglobin in the animal body. The approach to this problem has involved the determination of the rate of blood regeneration, after hemorrhage, upon diets containing different proteins and at different protein levels; it has involved a comparison of the relative effectiveness of complex foods like liver and blood and of simple purified rations; it has included a comparison of inorganic and organic iron compounds; it has entangled another inorganic factor, potassium, in the consideration of blood regeneration.

Brevity precludes any extensive discussion of the literature of blood regeneration. The question of the use of iron citrate for the formation of hemoglobin comes under the major consideration of the relative merits of "inorganic" versus "organic" iron for this process. The excellent reviews of Hall (1) and Meyer (2) leave one under the impression that both forms of iron can be used by the animal body in the synthesis of hemoglobin. The later work of Whipple (3) shows very conflicting results in the use of Blaud's pills for the formation of blood in the dog. No analytical data concerning the iron content of his basal diets have been furnished by Whipple, however. From the composition of his "breads" one must agree with Scott (4) that they probably contain ample iron. The addition of further iron could not be expected to throw much light upon the use of iron for hemoglobin formation. The same criticism applies to the experiments of Musser (5) whose mixed diets probably provided all the iron the animals needed without further additions. The work of Williams and Ets (6) shows very careful evaluation of the iron in their diets but their data show that they probably failed to exhaust the reserve iron in the bodies of their rats. As a result, their animals with and without iron in their diets recovered at equal rates. Recent papers

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of Hart (7) Mitchell (8) and McCollum (9) indicate wide differences in the absorption and use of different iron salts by the body.

Few studies of blood regeneration have been made which considered the differences in the quality of the protein. Jenks (10) believed this to be an important factor but her conclusions are based upon very small differences. Saunders (11) found casein more efficient in the maintenance of hemoglobin than wheat gluten. Liver has attracted widespread attention owing to its use by Minot and Murphy (12) in the treatment of pernicious anemia. There is no proof, however, that protein is the active principle concerned.

Various authors have postulated that the body must obtain in its food some pyrrole complex which it cannot synthesize but which is essential for the formation of hemoglobin. Abderhalden (13) suggested this idea but did not try to confirm it by experiments. It has also been suggested by Robertson (14). Hart (15) and co-workers in their nutritional anemia studies first considered such a pyrrole complex in cabbage to be essential for the formation of hemoglobin in the rabbit. In a later paper (7) this conception was reversed when it was discovered that the ash of the cabbage was equally effective. Hirasaw (16) found trytophane to exert a favorable influence upon blood regeneration in the rabbit. He attributed this to its heterocyclic ring. Saunders (11) has found that phaophytin will partially alleviate experimental anemia in the rat.

Few experiments have been devoted to the study of the influence of inorganic factors other than iron upon blood regeneration. Woronichin (cited by Hall (1)), and Sabelin and Wassilewsky (cited by Meyer (2)), maintain that both sodium and potassium chlorides influence the resorption of iron from the intestinal tract. No modern repetition of this work is known. In the course of our experiments we have devoted some attention to the element potassium.

Numerous studies to determine the relation of blood fed in the diet to that formed in the living animal have been reported. Socin (17), who was one of the earliest workers to employ a salt mixture whose composition was modeled after the ash of milk, fed mice a synthetic diet containing hemoglobin. But since all his mice except those fed egg yolk died in a very short period, he was unable to compare the relative effectiveness of hemoglobin and iron salts in their influence upon blood regeneration. Busch (18) believed that the iron from hemoglobin was absorbed in the digestive tract. His evidence consisted of a slight rise in the iron content of the urine after feeding hemoglobin. Cloetta (19) from studies of iron balances concluded that iron from blood which was fed could not be absorbed. Hausermann (20) fed hemoglobin to mice but obtained no more conclusive results than Socin. Abderhalden (21) using micro methods found iron absorbed when hemoglobin and hematin were fed to

rats. He also studied the hemoglobin formed and the growth rates of rats fed hemoglobin and hematin with milk. These showed some utilization but the values were quite inferior to those obtained upon a mixed diet. Barkan (22) has shown that very little of the iron is split from hemoglobin by acid equal in strength to that of the stomach or by digestion in vitro with pepsin or trysin. The tables of Whipple (3) show little if any relation between the hemoglobin of the diet and that formed in the body. We must conclude from past experiments that the value of blood of the diet for the formation of blood in the body is very doubtful.

The question of the relation of the fat of the diet to the rate of blood regeneration has frequently attracted attention but little experimental work has been done. Scott (4) has reviewed the literature. His own experiments did not show whether fat or some fat-soluble substance related to vitamin A was the essential factor. His animals showed the signs of a deficiency. Koessler, Maurer and Loughlin (23) concluded that the fat-soluble factor was closely connected with blood formation.

Animal technique and relation of liver and iron of diet to regeneration. Both the dog and the rat have been employed in these studies. Since the work of Verdeil (24) the dog has been widely used for experimental studies upon anemia. Modern improvements in the maintenance of dogs upon purified rations have rendered this species of greater value. The dog's chief virtue is in affording relatively large volumes of blood for manipulations. Many other factors, however, render the dog quite unsatisfactory. Few laboratories have at their disposal anything other than pound dogs. The ages of these are usually unknown. They display widely variable individual reactions in the regeneration of blood when they are fed purified rations. It is often of value to carry an experiment over a long period of time. To be comparable to a year's work upon the rat an experiment must include several years of work upon the dog. The excellent technique which Whipple (3) has developed for the dog, has enhanced its value, but has not been extended to include any long period of its life. Whipple has found this method effective for making comparative, short period feeding tests.

The rat has been used to excellent advantage by Scott (4), Mitchell (8), Williamson (6) and others in the study of blood formation. The advantages of this animal, inherent in his dietary habits have been amply extolled in other publications. It seems almost trite to state that the ultimate chemical units concerned with blood formation can only be determined when an animal is maintained upon synthetic rations of known compositions.

In all our experiments the animals were rendered anemic by the removal of blood from the heart. In the dog the technique described by E. W. Schultz (25) was employed for bleeding. No animal has been lost although

in some cases the heart has been punctured many times. In following recovery from an anemia in the dog, weekly samples were taken from the ear veins. In rendering an animal anemic, blood was removed until an ear sample showed a hemoglobin value of about one-half that of the normal animal. The relative efficacy of a diet for regeneration was judged by the time required for two or more complete recoveries upon the given diets. This is essentially the technique of Eger (26). For work with purified rations where the recovery is slow it is as satisfactory as the modified Kunkel (27) technique which is now employed by Whipple.

The technique employed with the rats must be described in greater detail not because it involves new principles but because the conventional method of handling the animals has been somewhat modified. Cardiac puncture of the heart must ultimately attain a wide use because of the ease of obtaining rat blood without injury to the animal. A perusal of the experimental data will show the large amounts of blood, twenty to fifty cubic centimeters, that have been withdrawn from individual rats in the course of one hundred to two hundred days.

Proper holding of the rats during the bleeding is very important. The animals must be very tame and used to handling. Two technicians are required, one to hold the animal and one to manipulate the syringe. One person can work alone when the animal is strapped to a board but this is much less satisfactory. The person holding the animal first places it with its back resting in his left hand. The thumb of this left hand should cross the chest of the animal just below the right foreleg and assist the left index finger in grasping the left forepaw of the rat. This affords a very effective means of holding the head of the animal, preventing his biting and at the same time eliminating choking. The right hand of the holder is used to grasp the hind legs. The animal is slightly stretched. Before the rat is placed in this position the hair is clipped from the left side of the thorax in the region of the ventricle.

After the animal is in position in the hands of the holder the "bleeder" carefully palpates the region of the left thorax to determine the position of the tip of the ventricle. We have found the tip of the small finger to be the most delicate in this work. This requires considerable practice but real expertness can be acquired. The needle most satisfactory for work upon the rat is 21 gauge and $\frac{3}{4}$ inch in length. Needles are sterilized in mineral oil and kept in this when not in use. After the beat of the ventricle has been definitely established the needle is pushed gently between the ribs, usually the fifth and sixth, at a position about one half inch from the sternum. The beat of the ventricle will be distinctly felt against the needle. A quick, short thrust is now employed to pierce the wall of the ventricle. Slight suction is maintained in the syringe. If the blood fails to flow it may often be started by slightly withdrawing the needle. During

the period in which the blood is being sucked into the syringe the needle is steadied by having the left hand holding the barrel of the syringe with the small finger of this hand resting upon the animal. The right hand operates the plunger. Either a 3 or a 5 cc. syringe is employed. The barrel is carefully lubricated with vaseline. Varying amounts of blood have been removed from individual rats at one time. The greatest withdrawals from large animals have been slightly in excess of five cubic centimeters although the usual amounts have been two cubic centimeters. After one or two lessons gentle rats will lie very quiet while the blood is being withdrawn and only flinch after the removal of a considerable volume. Working in this manner without any anesthesia is very satisfactory. The mortality rate is considerably less than for a similar operation under ether.

In our earlier experiments we employed the criterion of Williamson and Ets (6) in evaluating a diet for regeneration. This is the same as that of Eger (26). Blood is withdrawn from the rats until the hemoglobin value falls to about one-half the original. The time for recovery is then determined. This yields results that are satisfactory if the standards so clearly enunciated by Oerum (28) are observed, namely, the initial reserves of the rat must be exhausted before the recovery is started. After our first group of rat experiments we abandoned this technique of Eger and adopted a modification of that of Kunkel (27).

This modification involved an initial reduction of hemoglobin. After this the recovery was followed by weekly analyses of hemoglobin samples from the tail. As soon as the animal recovered, blood was removed again until a similar low level was reached. The weight of hemoglobin removed at each bleeding was determined quantitatively. This procedure was followed over long periods of time, usually one hundred to two hundred days. In the end all animals were allowed to recover. The weight of hemoglobin which had been removed and which represented the amount regenerated in a given time unit was known. This afforded a means of evaluating a given diet which was free from all considerations of blood volume. Furthermore since the experiments were in progress from three to seven months, the periods involved represent considerable portions of life spans.

In all our work the hemoglobin was determined by the method of Cohen and Smith (29). Values are expressed in the customary percentage units of Haldane. The latter terms a blood sample that has an oxygen capacity of 18.5 cc. per 100 mls. of blood a 100 per cent hemoglobin specimen. Erythrocytes were counted in a standard chamber using Gowers' solution for dilution. Our work assumes that the peripheral and central circulation have the same content of red blood cells. Such an assumption has been involved in most of the papers that have appeared recently in which rats

were used for the study of blood regeneration. From the statements of Donaldson (30) one must believe that the red cell count is higher in a sample from the tail than in one from the central circulation. To verify this we selected a group of rats. At weekly intervals samples were taken simultaneously from the tail and heart for red cell counts. This work was continued for three weeks yielding six counts from each rat. Complete data from eleven animals are reported in table 1. These demonstrated that there is no difference between the erythrocyte concentration of the peripheral and central circulation.[2]

TABLE 1

Relation of red cell counts of central and peripheral blood

(Millions per cmm.)

	NUMBER OF OBSERVATIONS	FIRST WEEK		SECOND WEEK		THIRD WEEK	
		Tail	Heart	Tail	Heart	Tail	Heart
Average	11	10.5	9.7	9.4	9.4	8.8	8.6
Maximum		10.8	10.5	10.7	10.5	9.9	9.9
Minimum		9.3	6.7	7.8	8.2	6.7	6.0

TABLE 2

Relation of hemoglobin level of central and peripheral blood

	NUMBER OF OBSERVATIONS	HEMOGLOBIN	
		Tail sample	Heart sample
		per cent	*per cent*
Average	23	99	96
Maximum		105	106
Minimum		94	85

In many cases the hemoglobin was determined upon samples from the tails of rats. Upon the following days similar analyses were often made upon samples from the heart in the process of evaluating the hemoglobin that was removed. This afforded extensive data for comparing the hemoglobin contents of the central and peripheral blood of the same animals. Only a typical portion of these data has been included in table 2. They show no differences in the hemoglobin value of blood taken from the heart and tail of the same animal, other than those due to experimental errors. We have found that this is only true, however, if samples are removed from the tail without much manipulation. Extensive handling of the tail

[2] The observations were made by Mr. Harold J. Harris.

causes the values to rise. We may conclude that a sample from the tail is representative of the circulating blood of the rat.

Our first experiments upon rats were undertaken to determine the use the rat could make of iron citrate for regeneration, to compare the rates of regeneration upon diets containing proteins from two different sources and to compare the rates of regeneration in the rat upon diets containing liver and upon purified, simple rations.

The following diets were prepared:

No. 1	per cent	*No. 2*	per cent
Coagulated Egg Albumen (14 per cent N)	30	Dried Liver Powder (10.7 per cent N)	39
Sucrose	46	Lard	15
Lard	20	Sucrose	42
Salt mixture	4	Salt mixture	4

No. 3

	per cent
Casein (12.7 per cent N)	34
Lard	20
Sucrose	42
Salt mixture	4

Osborne and Mendel's salt mixture was used. It was prepared in the usual manner with the omission of the iron citrate. Each of the diets was supplemented by an addition equal to a daily feeding of 3 to 5 drops of cod liver oil and 0.5 gram Vitavose (Squibb). This was fed separately three times per week. When iron citrate was added to the diet, it was made up in a solution of known strength. The desired volume was then added to the Vitavose from a pipette. This insured its consumption by the animal.

The components of the above diets were analyzed for iron by the methods of Foweather (33) after its limitations had been carefully studied through recoveries of known iron additions. The iron content of the constituents of our rations was:

	per cent Fe
Dried liver	0.086
Casein	0.009
Egg albumen	0.005
Vitavose	0.010

Vitavose was selected as a source of vitamin B, not because it is free from iron but because it has a lower content than the supplements usually fed. The yeast which is employed in this laboratory has the high iron content of 0.1 per cent. (Work in progress indicates that the utilization of this iron although it is "organic" is very doubtful.) The preparation of Harris contains 0.03 per cent and Vegex contains 0.03 to 0.04 per cent iron.

For the first experiments thirty young, adult rats were divided into five

groups and placed upon the following diets the composition of which has been given:

Group No.	*Diet No.*
1	1
2	1 plus iron citrate
3	2
4	3
5	3 plus iron citrate

Each rat consumed 2 mgm. of iron per day when iron citrate was added to the diet.

After the groups were placed upon these diets they were bled from the heart. The blood removed was thrown directly into N/10 HCl which had been placed in 5 cc. amounts in graduated centrifuge tubes (15 cc.). The volume removed at each bleeding was obtained by the difference in reading upon these tubes. An aliquot was employed for a hemoglobin determination. These data permitted the weight of hemoglobin, removed at each bleeding, to be determined. The animals were bled several times each week until the hemoglobin level fell to 50 to 75 per cent of the normal level. This reduction required four to six weeks. The animals were then allowed to recover. Sixty-five and ninety-four days after the opening of the experiment hemoglobin determinations were run upon tail samples. The results are shown in table 3. In this table we have shown rat number, weight of rat at beginning of the experiment, weight of rat at the end of the experiment, the volume of blood withdrawn, the number of cardiac punctures, the weight of hemoglobin withdrawn, the Hb per cent upon the 65th day and the Hb per cent upon the 94th day. Only data upon animals that lived in good condition throughout the experiment are recorded.

From these data we may conclude: *1*, it requires the withdrawal of about 1½ grams of hemoglobin to deplete the reserves of a normal adult rat; *2*, the rate of hemoglobin regeneration upon diets containing two different proteins, casein and albumin, is equal; *3*, iron citrate added to a synthetic ration low in iron hastens regeneration; *4*, upon a diet containing liver more hemoglobin must be withdrawn to render the animal anemic and its recovery is very rapid; *5*, adult animals can increase their weight in the course of such an experiment.

In the next group of experiments the technique and diets were modified to test a series of hypotheses which had been advanced by earlier workers. The first of these deals with the essential pyrrole complex for blood regeneration. For this purpose diets containing two different proteins were employed. Each of these proteins was fed at two different levels, a ten and a thirty-five per cent. Two groups of animals were fed each of the lower levels, one with and one without iron citrate. Two groups of animals were also placed upon the higher albumin level, one with and one

without iron. All the animals upon the higher casein diet received iron in order to have a larger number to compare with those receiving iron in natural form. These diets were designed upon the assumption that if protein contained an essential organic complex, it would promote blood regeneration more rapidly when fed at a higher level.

TABLE 3

	NUMBER OF OBSERVATIONS	WEIGHT		VOLUME	NUMBER OF WITHDRAWALS OF BLOOD	WEIGHT HEMOGLOBIN	HEMOGLOBIN	
		Beginning	End				Sixty-fifth day	Ninety-fourth day
Group one. "Albumin"—Diet 1								
				cc.		*grams*	*per cent*	*per cent*
Average	4	205	224	12.8	7	1.35	64	96
Maximum		248	250	14.9	8	1.49	72	102
Minimum		186	194	9.9	6	1.24	56	89
Group two. "Albumin plus iron citrate"—Diet 1								
Average	4	229	228	14.3	8	1.79	80	105
Maximum		270	255	19.0	9	2.03	93	115
Minimum		192	210	12.7	6	1.50	67	98
Group three. "Liver"—Diet 2								
Average	5	223	245	19.8	9	2.25	91	102
Maximum		234	250	24.9	10	2.78	100	107
Minimum		210	235	13.9	7	1.68	85	96
Group four. "Casein"—Diet 3								
Average	5	200	228	14.3	7	1.43	66	96
Maximum		230	255	19.9	10	1.69	82	107
Minimum		178	212	10.1	6	1.19	51	87
Group five. "Casein plus iron citrate"—Diet 3								
Average	5	197	246	12.9	7	1.42	91	108
Maximum		220	262	19.1	9	1.69	119	121
Minimum		164	220	10.1	5	1.20	75	104

In the second place we wished to expand the work of the first experiments upon iron citrate and to test its utilization by modified technique and different criteria. Thirdly, we wished to confirm the previous work upon liver and to compare the utilization of its iron with that of iron citrate. Fourthly, we wished to study the influence of the potassium of the diet upon regeneration.

The technique of feeding and bleeding was the same as that of the previous experiments. A slight modification in the sampling for the hemoglobin determination was introduced to shorten the time consumed in the work. Instead of taking an aliquot after the blood had been thrown into the graduated centrifuge tube containing hydrochloric acid, a drop was placed upon a crucible cover and this used for the hemoglobin determination. The volume of blood withdrawn was determined in the same manner as in the previous experiments.

Data upon sick animals or upon those that were killed after a short experimental period are not included.

The diets for these experiments had the following compositions:

No. 4	per cent	*No. 5*	per cent
Albumin (14.5 per cent N)	29	Albumin (14.5 per cent N)	9
Lard	20	Lard	20
Salt mixture	4	Salt mixture	4
Sucrose	47	Sucrose	67
No. 6		*No. 7*	
Casein (12.3 per cent N)	34	Casein (12.3 per cent N)	10
Lard	20	Lard	20
Salt mixture	4	Salt mixture	4
Sucrose	42	Sucrose	66
No. 8		*No. 9*	
Casein (12.3 per cent N)	22	Casein (12.3 per cent)	34
Liver (10.7 per cent N)	13	Lard	20
Lard	19	Bone ash	2.2
Salt mixture	4	Salt mixture (Karr)	1.8
Sucrose	42	Sucrose	42

Osborne and Mendel's salt mixture was used in all diets except no. 9. The iron citrate was omitted. The egg albumin was prepared by coagulating the commercial product after which it was dried and ground. The liver was prepared as in the previous experiment by cooking, drying and grinding. Karr's (31) salt mixture was employed in no. 9. It was augmented with 4 per cent KCl in the latter half of the experiment upon this diet. Vitavose, cod liver oil and iron were fed in the same manner as in the previous experiments.

Data upon diet, average weight, extent of the experiment in days, hemoglobin per cent at the beginning and end of the experiment, number of heart punctures, total volume of blood withdrawn in the course of the experiment, the weight of hemoglobin withdrawn and the weight of hemoglobin that a rat regenerates for each two hundred grams of body weight in each hundred days, have been placed in tables 4, 5 and 6. Tables 4 and 5 contain the studies upon diets 4 to 8 inclusive. Table 6 contains the data showing the influence of potassium upon regeneration. This table requires further explanation. In the two experimental groups

of animals originally placed upon diet 9, there were originally ten animals. Three of these were lost in the course of the experiment. The deviation in the salt mixture of no. 9 is due to the fact that this experiment was originally devised to parallel the similar work upon dogs. In this work the

TABLE 4

	DIET	NUMBER OF OBSERVATIONS	AVERAGE WEIGHT	EXTENT OF EXPERIMENT	HEMOGLOBIN		NUMBER OF WITHDRAWALS OF BLOOD	VOLUME REMOVED	WEIGHT HEMOGLOBIN REMOVED	WEIGHT HEMOGLOBIN REGENERATED PER 200 GRAMS PER 100 DAYS
					Beginning	End				
				"High albumin"						
				days	*per cent*	*per cent*				
Average	4	4	200	158	113	100	10	14.3	1.77	1.17
Maximum			230	198	123	108	14	16.2	1.94	1.61
Minimum			180	117	95	95	8	13.5	1.56	0.84
Average	4 and Fe	5	244	94	111	107	13	20.2	2.82	2.84
Maximum			290	132	128	118	17	23.9	3.44	4.62
Minimum			204	47	96	100	10	17.8	2.37	1.71
				"Low albumin"						
Average	5	4	223	192	108	91	6	9.8	1.13	0.53
Maximum			238	196	127	102	7	13.3	1.56	0.69
Minimum			210	191	94	73	5	7.3	0.77	0.35
Average	5 and Fe	4	202	167	117	102	23	27.5	3.69	2.29
Maximum			218	191	130	106	35	37.7	5.21	3.19
Minimum			194	96	104	102	12	19.9	3.04	1.63
				"High casein"						
Average	6 and Fe	9	229	134	107	104	15	27.1	3.58	2.50
Maximum			314	244	137	115	21	52.1	6.97	3.99
Minimum			170	61	94	91	6	16.6	2.19	1.48

rats were first fed for about three months with the salt mixture devised by Karr (31). This when employed with bone ash makes a diet quite low in potassium. The rate of regeneration was first measured without the potassium. At the end of an initial period, the exact length of which is given in the table, the salt mixture was augmented with potassium chloride.

The regeneration rate was measured again upon the same animals for a second period. This afforded a means of comparing the rate with and without potassium upon the same rats. The first period is numbered "1" in the table and the second "2".

Since it is quite possible that the rate of regeneration changes under the influence of constant hemorrhage and anemia it was thought desirable to select a miscellaneous group of animals which had been bled over con-

TABLE 5

	DIET	NUMBER OF OBSERVATIONS	AVERAGE WEIGHT	EXTENT OF EXPERIMENT	HEMOGLOBIN		NUMBER OF WITHDRAWALS OF BLOOD	VOLUME REMOVED	WEIGHT HEMOGLOBIN REMOVED	WEIGHT HEMOGLOBIN REGENERATED RAT PER 200 GRAMS PER 100 DAYS
					Beginning	End				
				"Low casein"						
				days	*per cent*	*per cent*				
Average.....	7	4	209	166	109	82	8	16.1	1.93	1.22
Maximum....			247	196	114	97	10	21.7	2.22	1.89
Minimum.....			186	95	100	60	7	15.1	1.88	0.78
Average.....	7 and Fe	5	222	140	109	105	15	27.0	3.82	2.88
Maximum....			282	249	121	119	21	36.9	5.45	4.29
Minimum.....			187	80	98	97	10	23.6	3.04	1.07
				"Liver—casein"						
Average.....	8	6	282	105	110	104	13	26.7	3.57	3.79
Maximum....			290	217	128	107	26	44.8	5.56	6.07
Minimum.....			162	34	100	98	4	10.9	1.29	1.77
Average.....	8 and Fe		221	102	112	107	17	30.2	4.31	4.29
Maximum....			270	215	120	120	25	55.1	7.53	6.42
Minimum.....			197	50	102	95	8	16.5	2.45	2.59

siderable periods of time, divide these periods into halves and compare the relative rates during the first and second periods when the diets were constant. Such selected data have been assembled in table 7.

Discussion of the data in tables 4, 5, 6 and 7 relation of protein level, liver and potassium to regeneration. The results incorporated in tables 4 and 5 show that equal amounts of hemoglobin are formed whether the protein of the diet is furnished by casein or albumin. They also show that differences in the protein level of the diet are without influence if the animal body

is supplied with adequate iron. Hence either a preformed pyrrole group is non-essential or the lowest protein level furnishes an adequate amount. This iron requirement is met by feeding iron citrate. If an outside source of iron is not provided in the diet and the animal is obliged to depend upon that which contaminates the diet a marked difference appears which might be regarded as a result of protein level. At the present time this may be considered a result of the differences in the amounts of iron which the animal obtains due to the iron contamination of the protein. A final

TABLE 6

Potassium in relation to regeneration

	DIET	NUMBER OF OBSERVATIONS	AVERAGE WEIGHT	EXTENT OF EXPERIMENT	HEMOGLOBIN		NUMBER OF WITHDRAWALS OF BLOOD	VOLUME REMOVED	WEIGHT HEMOGLOBIN REMOVED	WEIGHT HEMOGLOBIN REGENERATED PER 200 GRAMS PER 100 DAYS	PERIOD
					Beginning	End					
				days	*per cent*	*per cent*					
Average.....	9 and Fe	3	221	122	103	106	14	21.7	2.96	2.22	1
Maximum.....			254	122	105	108	19	22.6	3.18	2.39	1
Minimum.....			190	122	100	103	10	19.9	2.77	2.07	1
Average.....	9, Fe and K	3	232	103	106	100	12	14.3	1.83	1.59	2
Maximum.....			252	125	108	105	14	17.3	2.25	1.71	2
Minimum.....			210	81	103	93	10	9.6	1.24	1.36	2
Average.....	9	4	214	103	110	106	7	13.9	1.78	1.55	1
Maximum.....			232	105	118	114	10	17.1	2.45	2.15	1
Minimum.....			210	101	104	104	4	12.3	1.40	1.28	1
Average....	9 and K	4	235	119	106	109	9	19.5	2.47	1.78	2
Maximum.....			236	125	107	121	12	26.8	3.47	2.36	2
Minimum.....			220	102	104	96	6	12.0	1.56	1.06	2

conclusion must await studies using proteins that have been purified until they are practically iron free. The results from the liver diet show that liver renders the ordinary diet superior to one in which it is omitted even if it furnishes but part of the protein. Iron added to a liver diet is without influence upon regeneration.

Table 6 involves a comparison of the rate of regeneration in the same animals fed a diet that is only varied by the presence or absence of potassium. Potassium exerts an influence even when the diet contains ample

iron. When the diet is exceedingly low in iron the influence becomes very marked. Table 7 in which data have been assembled for animals upon various diets in order to determine if there is a change in regeneration rate when an animal is kept anemic for a considerable period, shows that in the second half of any given period the rate usually decreases. On the other hand if potassium is omitted from the diet during the first half of a period and included during the second half, the latter period shows a marked increase in rate of blood regeneration if the diet is deficient in iron.

A decrease in the rate of recovery during a long anemic period shows that the usual criteria of blood regeneration may be false. When the cell count and hemoglobin of an anemic animal have returned to normal it is said to be recovered. The recovery is probably only apparent in many cases since the reserves may remain very low. The contrast in level of

TABLE 7

Time in relation to regeneration

	NUMBER OF OBSERVATIONS	AVERAGE WEIGHT	EXTENT OF EXPERIMENT	HEMOGLOBIN		WEIGHT HEMOGLOBIN REMOVED	WEIGHT HEMOGLOBIN REGENERATED PER 200 GRAMS PER 100 DAYS	NUMBER OF PERIOD
				Beginning	End			
			days	*per cent*	*per cent*			
Average	8	222	102	105	106	2.39	2.71	1
Maximum		280	158	128	119	4.32	3.95	1
Minimum		155	82	94	98	1.73	1.38	1
Average	8	251	92	106	105	1.89	1.63	2
Maximum		320	125	119	110	3.21	3.56	2
Minimum		195	74	98	99	0.34	0.41	2

reserves is the great differences in amounts of blood which must be removed to reduce the hemoglobin content of an animal that has never been anemic and one that has just recovered, to a similar low blood level.

The following experiments were designed for the purpose of comparing the rates of regeneration upon diets containing blood and liver as sources of iron with the rates obtained upon diets with casein at two different levels as the source of protein and iron citrate as the source of iron. At the same time a series of studies was made upon diets with widely different fat content to determine the influence of this factor.

All these diets were so constituted that they contained adequate casein for the normal maintenance of the animal. In both the blood and liver diets these extra components were superimposed upon the casein. The blood and liver served as sources of iron since no iron was included in the salt mixture. In the studies with fat as the variable three different fat

levels were employed, one very high, one very low and one intermediate. The other parts of the diet were so established as to insure equal intakes of nitrogen and salts upon each of the three.

No. 10

	per cent
Casein (12.3 per cent N)	10.2
Liver (10.7 per cent N)	28
Sucrose	39
Lard	19
Salt mixture	4

No. 11

	per cent
Casein (12.3 per cent N)	10.2
Blood (15.5 per cent N)	20
Sucrose	46
Lard	20
Salt mixture	4

No. 12

Casein (12.3 per cent N)	18
Starch	53
Lard	23
Salt mixture	4

No. 13

Casein (12.3 per cent N)	25
Lard	69
Salt mixture	6

No. 14

	per cent
Casein (12.3 per cent N)	14
Starch	82.8
Salt mixture	3.2

These diets were supplemented with cod liver oil and Vitavose in the same manner as the preceding ones. The blood of no. 11 was commercial dried beef blood which was further dried and ground.

The same technique of rendering the animals anemic was employed as that used in the preceding experiments. The chief modification consisted in feeding the animals all the same diet until they had been rendered anemic. They were fed no. 7, the low protein diet without iron addition. This procedure made it possible to reduce the animals upon a diet which had previously been shown to permit the regeneration of very little hemoglobin. Comparable values for the hemoglobin regenerated are obtained within the group but they are somewhat lower than those of the preceding experiments.

Discussion of data in tables 8 and 9. Table 8 shows that the rate of regeneration is practically equal upon the three diets containing high casein content, a low casein content and a considerable amount of blood. The diet containing cooked dried liver in place of the blood is quite superior. It shows that the blood content of liver is not the active agent in the promotion of blood regeneration. Iron was furnished in both the casein diets. These repetitions confirm the previous experiments in showing that the rate of blood regeneration is independent of the protein level if the animal receives adequate iron.

Table 9 shows the rates of regeneration upon the diets of various fat

levels. These would lead one to conclude that there is no relation between the fat content of the diet and the rate of blood regeneration.

STUDIES UPON DOGS. The feeding technique for dogs which has been

TABLE 8

	DIET	NUMBER OF OBSERVATIONS	AVERAGE WEIGHT	EXTENT OF EXPERIMENT	HEMOGLOBIN		NUMBER OF WITHDRAWALS OF BLOOD	VOLUME REMOVED	WEIGHT HEMOGLOBIN REMOVED	WEIGHT HEMOGLOBIN REGENERATED PER 200 GRAMS PER 100 DAYS
					Beginning	End				
				"Low casein"						
				days	*per cent*	*per cent*				
Average	7 and Fe	5	183	137	112	99	17	17.2	2.22	1.77
Maximum			205	172	117	202	22	22.3	3.38	2.24
Minimum			140	109	100	94	12	9.1	1.18	1.48
				"High casein"						
Average	6 and Fe	5	178	109	105	101	14	15.4	1.82	1.83
Maximum			215	173	115	107	26	26.8	3.09	2.08
Minimum			172	69	94	94	9	8.6	0.74	1.33
				"Liver"						
Average	10	4	216	137	110	101	18	24.1	3.02	2.28
Maximum			250	173	120	114	24	28.4	3.57	3.23
Minimum			155	75	102	92	12	20.1	2.65	1.47
				"Blood"						
Average	11	5	205	123	107	102	13	14.0	1.78	1.53
Maximum			232	158	125	107	18	18.3	2.37	2.48
Minimum			158	83	98	94	10	9.2	1.17	0.83

developed by Karr (31), Cowgill (32) and earlier workers was used in these experiments upon blood regeneration. The following diets were employed:

	No. 15	*No. 16*	*No. 17*
	per cent	*per cent*	*per cent*
Casein	34	23	11
Lard	20	20	20
Cod liver oil	2	2	2
Sucrose	40	40	40
Bone ash	2.2	2.2	2.2
Salt mixture	1.8	1.8	1.8
Dried liver	0	11	23

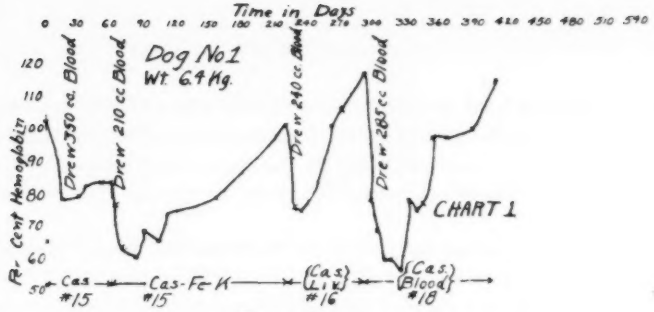
	No. 18	*No. 19*
	per cent	*per cent*
Casein	23	11
Blood	11	23
Lard	20	20
Sucrose	40	40
Cod liver oil	2	2
Bone ash	2.2	2.2
Salt mixture	1.8	1.8

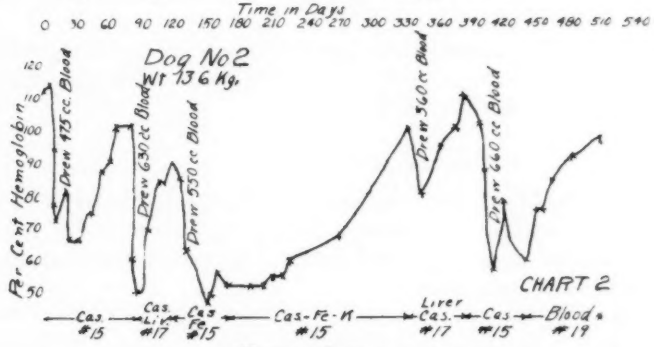
Vitavose was fed separately at the rate of 0.6 gram per kgm. per day. Karr's salt mixture was employed. In cases where potassium is indicated the salt mixture was augmented by 4 per cent of its weight of KCl.

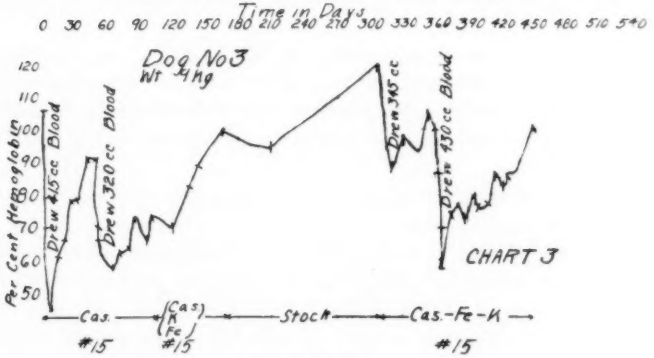
TABLE 9

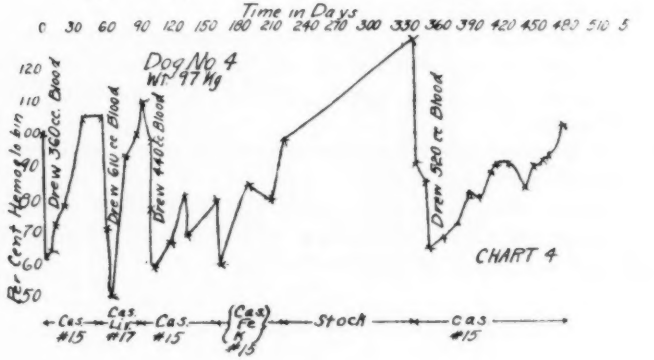
	DIET	NUMBER OF OBSERVATIONS	AVERAGE WEIGHT	EXTENT OF EXPERIMENT	HEMOGLOBIN		NUMBER OF WITHDRAWALS OF BLOOD	VOLUME REMOVED	WEIGHT HEMOGLOBIN REMOVED	WEIGHT HEMOGLOBIN REGENERATED RAT PER 200 GRAMS PER 100 DAYS
					Beginning	End				
					"Moderate fat"					
				days	*per cent*	*per cen*				
Average	12 and Fe	4	176	118	107	99	17	18.0	2.30	2.30
Maximum			200	145	119	102	23	30.4	3.75	2.80
Minimum			147	56	89	95	10	13.0	1.56	1.83
					"High fat"					
Average	13 and Fe	5	199	115	108	106	11	16.2	2.05	1.80
Maximum			215	134	119	119	17	23.6	2.71	2.31
Minimum			185	74	95	94	7	7.4	0.99	1.40
					"Low fat"					
Average	14 and Fe	5	210	128	106	106	12	15.8	1.97	1.50
Maximum			240	134	114	114	16	16.8	2.15	1.66
Minimum			180	104	100	100	9	13.1	1.70	1.11

The animals were rendered anemic by removal of blood by cardiac puncture. The rate of regeneration was followed by examining samples taken from the ear veins. Erythrocyte counts were made but have not been recorded here with the data since the values for hemoglobin show all the essential results. The data from about two years' work upon four dogs have been incorporated in graphs 1 to 4.


Time in Days
0 30 60 90 120 150 180 210 240 270 300 330 360 390 420 450 480 510 540
Dog No 1
Wt 6.4 Kg.
Per Cent Hemoglobin
120 110 100 90 80 70 60 50
Drew 350 cc. Blood
Drew 210 cc Blood
Drew 240 cc Blood
Drew 285 cc Blood
CHART 1
Cas #15
Cas-Fe-K #15
(Cas. Liv) #16
(Cas. Blood) #18


Time in Days
0 30 60 90 120 150 180 210 240 270 300 330 360 390 420 450 480 510 540
Dog No 2
Wt 13.6 Kg.
Per Cent Hemoglobin
120 110 100 90 80 70 60 50
Drew 475 cc. Blood
Drew 630 cc Blood
Drew 530 cc Blood
Drew 360 cc Blood
Drew 660 cc Blood
CHART 2
Cas #15
Cas Liv #17
Cas Fe #15
Cas-Fe-K #15
Liver Cas. #17
Cas #15
Blood #19


Time in Days
0 30 60 90 120 150 180 210 240 270 300 330 360 390 420 450 480 510 540
Dog No 3
Wt 11 Kg
Per Cent Hemoglobin
120 110 100 90 80 70 60 50
Drew 415 cc Blood
Drew 320 cc Blood
Drew 345 cc
Drew 430 cc Blood
CHART 3
Cas. #15
(Cas. K Fe) #15
Stock
Cas.-Fe-K #15


Time in Days
0 30 60 90 120 150 180 210 240 270 300 330 360 390 420 450 480 510 5
Dog No 4
Wt 9.7 Kg
Per Cent Hemoglobin
120 110 100 90 80 70 60 50
Drew 360 cc. Blood
Drew 610 cc Blood
Drew 440 cc Blood
Drew 520 cc Blood
CHART 4
Cas #15
Cas Liv #17
Cas. #15
(Cas Fe K) #15
Stock
Cas #15

The hemoglobin level is plotted on the ordinate while time in days is plotted upon the abscissa. The periods of bleeding and feeding the various diets are indicated. The steepness of the curve shows the speed of regeneration. The following conclusions may be drawn from these data upon dogs:

Upon a synthetic diet of casein and an adequate salt mixture containing iron there is a very slow regeneration.

In the absence of iron there is very little regeneration after the reserves of the body are exhausted.

Liver in the diet causes a marked acceleration of the rate of regeneration.

Dried blood in the diet is much less effective in promoting blood regeneration than a similar quantity of dried liver.

SUMMARY

Rats and dogs have been rendered anemic through removal of blood by cardiac puncture. The rate of hemoglobin regeneration has been studied upon a number of purified diets. The following conclusions have been drawn from these studies:

The blood of the central circulation has the same hemoglobin content and the same erythrocyte content as that of the peripheral circulation in the rat.

The rat can use iron citrate quite effectively for blood regeneration.

The rat is able to synthesize blood quite effectively upon a standard synthetic casein diet while the dog does so only with considerable difficulty.

Dried liver promotes very rapid blood regeneration in the rat as well as the dog.

Dried blood can be partly used for the formation of blood but its value cannot approach that of dried liver.

The rate of blood regeneration in the rat is independent of the protein level within wide limits providing there is adequate protein for the maintenance of the body. This indicates that a preformed pyrrole is either non-essential or furnished in ample amounts when the animal is allowed 10 per cent protein in its diet.

Recovery from anemia may often be merely apparent because the blood picture may indicate a recovery while the reserves remain exhausted.

Albumin and casein seem equally effective in promoting blood regeneration in the rat.

If the diet is adequate in every other respect the rate of regeneration is independent of its fat content.

These studies contribute some reason for questioning whether the synthetic rations which are satisfactory for the growth and maintenance of the rat may be suitable when fed to the dog.

Dried blood is not toxic to the adult rat since this animal can be maintained upon a diet which contains considerable amounts of it for long periods. During this time the diet contained casein as an adequate source of protein.

The writer's sincere thanks are due to Prof. Layfayette B. Mendel for his suggestions and criticism throughout this work.

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A STUDY OF THE SENSORY AREAS ELICITING THE SWALLOWING REFLEX

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Since the introduction of the use of iodized oil by Sicard and Forestier in 1922 (1) in clinical medicine, extensive use has been made of this radio-opaque substance in accurately outlining the bronchial tree in the roentgenographic diagnosis of certain bronchopulmonic lesions. A number of different methods have been described and are in common use for the administration of this iodized oil, but perhaps one of the most satisfactory methods, because it obviates the use of the bronchoscope and other intratracheal instruments, is the one (2) by which the oil is allowed to descend supraglottically into the trachea, aided by gravity and aspiration, after the widespread application of cocaine to the pharyngeal regions.

The well-known demonstration of the reflex character of the deglutory act and its dependence on afferent impulses from different areas in the mouth and pharynx by Wassilieff (3) naturally suggests that the administration of cocaine to the pharyngeal regions in the administration of lipiodal owes its effectiveness to the depression or elimination of the swallowing reflex, and that this has been brought about by the paralysis of the sensory areas in the natural food passages. The considerable impetus that has been given to the use of iodized oil in studies of pulmonary diseases by the supraglottic method, has therefore aroused the question if there might not be a specific area, or a few limited areas, stimulation of which alone is uniformly responsible for inducing the swallowing reflex.

A study undertaken to determine the relative ease with which the swallowing reflex is elicited from the various regions in the throat of man should possess not only general physiological interest but clinical significance as well. Should specific areas be found which are always responsible for the initiation of the swallowing reflex, the amount of cocainization could be greatly reduced. This would be a very desirable thing in view of the not infrequent occurrence of cocaine intoxication following the application of this drug to mucous surfaces. We wish to thank Dr. A. J. Ochsner, now of Tulane University, for suggesting this problem.

As far as known no detailed observations on man have been made with reference to the most sensitive sites in the throat for inducing the swallow-

ing reflex. In 1888 Wassilieff (3) was unable to find on himself a single area which if stimulated alone would invariably elicit the swallowing reflex, but he did succeed in entirely abolishing the reflex by swallowing a sponge saturated with a solution of cocaine and then withdrawing it with a string.

Studies have been made on a number of species of mammals in attempts to determine precise areas in the pharnygeal regions at which the afferent impulses originate, and the stimulation of which is most effective in initiating the swallowing reflex. Thus, according to Kahn (4), in the rabbit the most sensitive area in this respect is the soft palate in the region between the posterior portion of the hard palate and the tonsils. In the dog the chief sensory area appears to be located on the posterior pharyngeal wall. The same is true in the case of the cat. It is of interest with reference to what is to follow in this paper that Kahn found that in monkeys the chief area is along the palatine arch in the region of the tonsils.

EXPERIMENTAL. In the present study one hundred and twenty-six students at the University of Wisconsin were used as subjects. These were informed as to the purpose and nature of the experiments and their coöperation secured. Different areas of their throats were stimulated by the light application of blunt glass rods; with fine jets of water directed against localized areas; or with the direct application of faradic stimuli. After many trials the latter two methods were discarded. It was difficult to attribute a specific effect to the jets of water because it was impossible to keep water in definitely restricted areas. The faradic method of stimulation was objectionable because of the difficulty in separating the mechanical from the electrical effect, and because of the pain produced by this form of stimulation.

The subjects were instructed to swallow only when caused to do so in response to the stimulus, but not to inhibit the impulse to swallow. Various areas in the throats were stimulated, first by the application of a light touch with the glass rod to a single area. Then if no reflex occurred the rod was moved back and forth over a slightly larger area, more pressure being applied so as to stimulate more successive spots. When the application of the stimulus to any given area initiated the deglutory reflex a record of this fact was made on a separate mimeographed sheet containing a diagram of the accessible pharyngeal regions. The relative ease with which different areas responded to stimulation, or the failure of an area to respond, was noted in every case and appropriate individual records kept. It was found that there is very great variation in different individuals with respect to the ease with which the reflex can be started. These variations ranged all the way between those cases in which even the slightest or gentlest pressure applied to a given area in the pharynx would cause unmistakable swallowing movements, to those cases in which the throats

were normally so insensitive that they would endure considerable mechanical irritation, even to the extent that locking hemostatic forceps into them caused no marked effect as far as pain or the swallowing reflex was concerned.

Compilation of the data showed that there is apparently no single area which will in all individuals invariably cause the swallowing reflex when mechanically stimulated. Great variations were also found to exist in

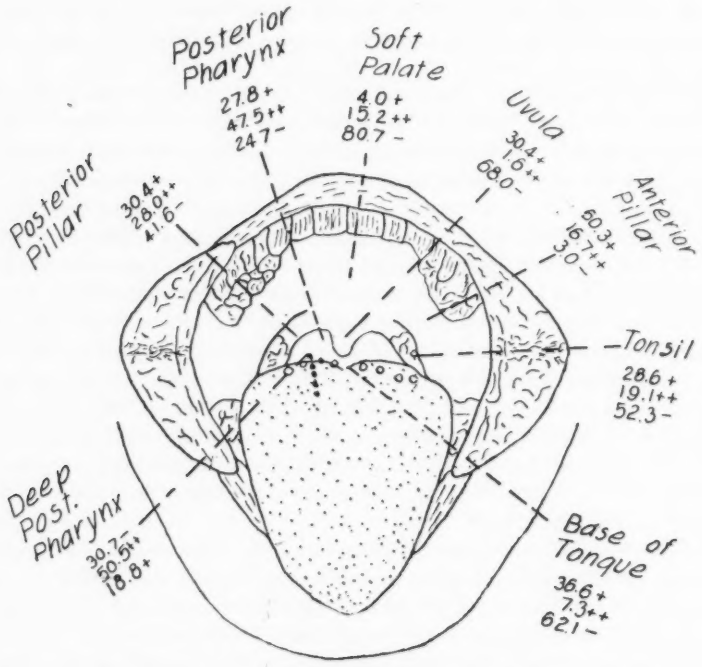

Fig. 1. Showing the effect of stimulating different areas in the pharynx.
The plus (+) sign indicates a swallowing reflex in response to a light stimulus over a small area. The double plus (++) sign indicates a swallowing reflex which occurred only in response to a stronger stimulation over a larger area. The minus sign (−) indicates no response. The figures represent percentages.

the different areas with regard to their susceptibility in causing swallowing in different individuals. The results of this study on 126 individuals are summarized in figure 1. In spite of the variations just mentioned, one thing of great importance brought out by this experiment is that of all the regions studied, the pillars, particularly the anterior, are most sensitive,

and the soft palate and uvula least sensitive. The posterior wall of the pharynx was found to be able to arouse the reflex upon stimulation in about the same percentage as the anterior pillars, a stronger stimulus however being required. In 77 per cent of the individuals it was possible to initiate the swallowing reflex by the stimulation of the anterior pillars. That only 23 per cent of the cases which failed to respond upon stimulation of this area, as opposed to the 80.8 per cent and 68 per cent failures to respond when the soft palate and the uvula respectively were stimulated, is regarded as evidence that the anterior pillars of the fauces bear a more important relationship to the swallowing mechanism. One might have expected that the sinus tonsillaris would yield a larger percentage of positive reactions because of its proximity to the anterior and posterior pillars than was found to be the case. A possible explanation for this fact is that due to scar tissue formation following tonsillectomy, in a large number of cases, this area became less sensitive to mechanical stimulation.

The study was extended to determine, if possible, the effect of painting different limited areas in and about the pharynx with cocaine. Seventy-two different individuals were used in these tests. Of these seventy-two individuals, twenty-four persons were given two or more applications of cocaine hydrochloride which was always used in amounts of $\frac{3}{4}$ cc. of a 10 per cent solution. A total number of one hundred sixty-five applications was made. In a considerable number of cases 1:1000 adrenalin chloride was added to the cocaine solution in the proportion of one drop to 10 cc. to localize further the anesthetized area which was being painted with a small brush or cotton swab. Even by taking this precaution, it was possible that, because of untimely swallowing or gagging, some of the cocaine produced its effect beyond the original site of application. Where this was known to have occurred the data for that particular case were discarded. The resultant impediment in swallowing, chiefly evidenced by difficulty in raising the thyroid cartilage, and by subjective symptoms, was noted. Those individuals who received more than one application of cocaine were asked to make a mental note to the degree of difficulty they had encountered in swallowing with each application and were asked to compare the effects in each case. Compilation of the records furnished the data for table 1.

Without exception, in these individuals, the application of cocaine to the anterior pillars caused a definite impairment to swallowing. Nine of the forty-nine individuals whose anterior pillars had been cocainized found it impossible to swallow when these regions were stimulated until after the effects of the cocaine had worn off. Twenty individuals of this group suffered marked impairment to the reflex and found it all but impossible to voluntarily swallow. It is significant that the swallowing

reflex was completely abolished only in that group of individuals whose anterior pillars had been painted.

Cocainization of the posterior pillars was effective in 54.5 per cent of the cases in impairing the swallowing act. Inspection of table 1 shows that the extent of the effect when this area was cocainized was noticeably less than was the case of the anterior pillars.

That the uvula and soft palate apparently bear little relation to the swallowing mechanism is evidenced by the fact that only in about 14 per cent of the cases did cocainization of this area interfere with the swallowing act. That the percentage in this particular case is as high as it is may perhaps be due to the fact that some of the cocaine, instead of exerting its effect at the site of its original application alone, ran down over the very sensitive anterior pillars and produced its effect there.

When the sinus tonsillaris was cocainized there was a resultant impair-

TABLE 1

Showing the effects of the application of cocaine to different areas in the pharynx

−, No impairment to swallowing. +, Mild impairment to swallowing. ++, Moderate impairment to swallowing. +++, Marked impairment to swallowing. ++++, Very marked impairment to swallowing.

The figures indicate the number of subjects in which the particular effects occurred.

AREA PAINTED	−	+	++	+++	++++
Anterior pillars	0	9	11	20	9
Posterior pillars	15	9	6	3	0
Uvula and soft palate	19	3	0	0	0
Sinus tonsillaris	1	13	4	1	0
Posterior wall of pharynx	17	8	2	0	0
Base of tongue	11	4	0	0	0

ment in swallowing in eighteen of the nineteen cases. However, the degree of impairment was noticeably less than in the cases of the pillars. How much of the effect produced by the cocain in this general area could be attributed to the spreading of the drug to the neighboring pillars is, of course, difficult to say.

Cocainization of the posterior wall of the pharynx and of the base of the tongue was relatively ineffective as far as impairment of the swallowing reflex is concerned. Two individuals whose posterior wall of the pharynx had been cocainized suffered a moderate impairment to swallowing, but cocainization was entirely without effect in 63 per cent of the cases. Cocainization of the base of the tongue was without effect in 73 per cent of the cases, the remaining 27 per cent showing only a mild impairment to swallowing.

SUMMARY

From the data presented it appears that the areas in the pharynx mechanical stimulation of which will initiate the swallowing reflex vary considerably in different individuals, and that to no single area can be ascribed the exclusive power of causing the swallowing act. The anterior pillars of the fauces, however, appear to be notably effective in instituting the swallowing reflex as evidenced by the fact that a larger percentage of individuals responded to a light stimulus to this area than to any other area studied. The posterior pharynx and the posterior pillars were next in order of sensitiveness. The uvula and soft palate were found to be relatively very insensitive as far as initiating the swallowing reflex is concerned.

Cocainization of different areas in the pharynx bears out the above conclusions and shows that the anterior pillars of the fauces are particularly sensitive to the effects of the drug as far as impairment of the swallowing act is concerned. The posterior pillars and the sinus tonsillaris are also noticeably affected by cocaine but to a lesser extent than the anterior pillars. There is very little impairment to swallowing when the uvula, soft palate, and the base of the tongue and posterior wall of pharynx are cocainized.

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CHANGES IN THE SEDIMENTATION RATE OF THE ERYTHROCYTES AND IN THE PLASMA PROTEINS FOLLOWING PROLONGED CHLOROFORM ADMINISTRATION TO THE DOG

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Whipple and his co-workers (1), (2), (3) have demonstrated that the liver is the organ chiefly concerned with the formation of fibrinogen, and that prolonged chloroform anesthesia interferes with this function to such an extent that only an indeterminable trace of fibrin can be demonstrated in the plasma on the second or third day after anesthetization. Various investigators who have studied the sedimentation rate of the red blood cells have held that this phenomenon is related to the amount of fibrinogen present in the plasma—an increased fibrinogen leading to an increased rate of settling. The object of the present study was to determine any relationship which may exist between the settling rate of the erythrocytes and the plasma fibrinogen, when the latter had been altered by chloroform administration to experimental dogs. The sedimentation rate, the plasma albumin, globulin, and fibrin, and the hematocrit were studied quantitatively, and observations were noted on the bile pigment changes in plasma and urine, as well as on the lipoid variations in the plasma.

All dogs were given chloroform according to the technic used by Whipple and Hurwitz (3). Anesthesia was continued for from one and one-quarter to two and one-half hours, depending upon the size and condition of the animal. A control blood was taken from the heart of each animal immediately before the anesthesia was begun and thereafter at daily intervals during the continuance of the experiment. Two samples of blood were taken at each puncture without removing the needle. First, 2 cc. of blood were collected under oil using heparin as an anticoagulant for the determination of the sedimentation rate according to a method which has recently been developed in this laboratory (4); another 8 cc. sample was then taken over two drops of saturated potassium oxalate solution for determination of the plasma proteins after the method of Wu (5).[1] Hema-

[1] The fibrinogen in the oxalated plasma is precipitated as fibrin by the addition of calcium ions according to this method. In order not to confuse the identity of the two proteins, fibrinogen and fibrin, we shall always refer to the unprecipitated native protein in the plasma as fibrinogen, for the purposes of this paper.

tocrit readings were made after centrifuging the oxalated bloods in 15 cc. graduated centrifuge tubes for 20 minutes at 2500 R.P.M., and also by whirling the heparin samples in the special sedimentation tubes at the same speed for the same length of time. Oxalate causes diminution in the size of the individual cells with a corresponding increase in the amount of plasma per unit volume of blood. On the other hand, we have found that heparin, in the amounts necessary to prevent coagulation, does not disturb the water balance between cells and plasma, and may therefore be used for accurate measuring of the cell volume percentage by the hematocrit method. Since there were large and rather inconsistent variations in the fluid exchange in the different oxalated samples and since we had no method for determining fibrin upon heparinated plasma, it was decided to reduce the fibrin figures to their normal undiluted values by calculating them in terms of heparin-plasma volume. Assuming with Foster and Whipple (2) that the cells contain no fibrinogen and that the fluid drawn from them by the potassium oxalate is fibrinogen-free, the concentration of fibrin in the undiluted (heparinated) plasma can be determined by a simple proportion:

$$A : B :: X : Y$$

when A = oxalate plasma volume per cent
B = heparin plasma volume per cent
X = heparin fibrin—(to be calculated)
and Y = oxalate fibrin—(as determined)

All heparin-fibrin values in the tables and text were computed from this equation.

Curves representing the sedimentation rate of each blood were plotted from the readings made upon each sample, in millimeters settled per unit of time. A uniform time of one hour was chosen for comparison in the day-to-day changes of rate, since at this time the curves for the separate blood samples were most frequently rising uniformly, being damped neither by primary agglutination nor by packing. Whenever an actual reading was not made at the end of sixty minutes an interpolated value was obtained from the curve.

From a series of control experiments, it appeared that the sedimentation rate of many apparently normal dogs was very slow as compared with normal human beings. The normal fibrin content of dog's plasma was approximately 0.30 per cent, although individual variations were rather wide. This latter observation agrees with that of Whipple (1).

Dog 1. Female. Weight 12 lbs. Anesthetized with chloroform June 29, 3:00–5:30 p.m.

SAMPLE NUMBER	TIME	MILLIMETERS SETTLED IN 1 HOUR	PLASMA				HEPARIN HEMATOCRIT	REMARKS
			Fibrin	Albumin	Globulin	Total protein		
	1926							
1	June 29, 2:30 p.m.	1.8	0.33	4.29	2.00	6.62		Before anesthesia
2	June 30, 1:45 p.m.	0.4	0.15	4.52	1.78	6.45	37.7	Drowsiness; diarrhea. Urine and plasma more colored
3	July 1, 2:30 p.m.	Not enough to read	Indeterminable	4.58	2.01	6.59		Extreme drowsiness; diarrhea. Urine and plasma bright orange in color

Dog died during night of July 1–2.

Dog 2. Male. Weight 14 lbs. Slight distemper. Chloroform given August 2, 10:30–11:45 a.m.

SAMPLE NUMBER	TIME	MILLIMETERS SETTLED IN 1 HOUR	FIBRIN CALCULATED FROM HEPARIN HEMATOCRIT	PLASMA				HEPARIN HEMATOCRIT	OXALATE HEMATOCRIT	REMARKS
				Fibrin	Albumin	Globulin	Total protein			
	1926									
1	Aug. 2, 10:15 a.m.	26.0	0.62	0.55	4.55	2.37	7.47	40.0	32.2	Plasma clear straw color
2	Aug. 3, 9:00 a.m.	11.2	0.51	0.47	4.10	2.17	6.74	43.2	38.8	Plasma slightly yellow. Loss of appetite
3	Aug. 4, 9:00 a.m.	8.0	0.16	0.14	3.70	2.52	6.36	43.6	36.5	Plasma bright yellow. Urine yellow. Drowsy
4	Aug. 5, 9:00 a.m.	1.3	0.12	0.11	3.76	2.43	6.30	43.6	38.5	Plasma bright yellow and rich in lipoids. Urine bright yellow. Eating again
5	Aug. 6, 9:00 a.m.	4.0	0.25	0.24	4.05	2.20	6.49	41.7	39.7	Plasma and urine bright yellow
6	Aug. 7, 1:30 p.m.	10.0	0.34	0.33	4.07	2.24	6.64	40.0	38.0	Plasma and urine yellow
7	Aug. 9, 10:15 a.m.	36.0*	0.42	0.41		2.50		35.7	34.5	Plasma clear but slightly yellow
8	Aug. 10, 3:00 p.m.	43.8*	0.61	0.59	3.46	2.74	6.79	31.6	29.8	Plasma clear straw color

* Packing occurred, making these values somewhat lower than if the settling had not been impeded. The dog was given 40 cc. of 20 per cent glucose solution subcutaneously twice on August 2 and once on August 3 and August 4. After August 10, the dog became increasingly weak and was put to death on August 17.

Dog 3. Male. Weight 19.5 lbs. Given chloroform July 13, 1:30–3:00 p.m. See note

SAMPLE NUMBER	TIME	MILLIMETERS SETTLED IN 1 HOUR	PLASMA				HEPARIN HEMATOCRIT	OXALATE HEMATOCRIT	REMARKS
			Fibrin	Albumin	Globulin	Total protein			
	1926								
1	July 13, 1:30 p.m.	34.0*	0.55	3.33	1.56	5.44	32.4	22.7	Plasma clear straw color
2	July 14, 10:30 a.m.	23.0	0.34	3.23	1.62	5.19	30.1	22.7	Plasma clear straw color
3	July 15, 10:30 a.m.	10.0	0.17	3.37	1.97	5.51	32.5	26.0	Plasma slightly yellow
4	July 16, 1:45 p.m.	6.8	0.20	2.88	1.18	4.26	31.0	22.0	Plasma yellow and rich in lipoids. Urine orange
5	July 17, 10:00 a.m.	5.5	0.19	2.90	1.34	4.43	35.6	24.3	Plasma yellow and cloudy. Urine yellow
6	July 19, 10:00 a.m.	14.1	0.29	2.12	1.41	3.82		26.4	Plasma pale yellow. Urine bright yellow
7	July 24, 10:30 a.m.	34.5*	0.45					28.6	Plasma normal color and clear. Urine pale yellow

Note: This dog had been given Witte's peptone previously in another experiment. Peptone intoxication increases the sedimentation rate and the plasma fibrin.

* Packing made these values somewhat less than they would otherwise have been. Dog given 50 cc. of 20 per cent glucose solution subcutaneously on July 13th at 5:00 p.m.

In dog 3, the diminishing albumin, globulin and total protein percentages are probably due to the peptone poisoning which had been induced previously. This animal with an abnormally rapid sedimentation rate and with an unusually high plasma fibrin before the administration of chloroform was employed because it was desired to see whether the fibrinogen and the sedimentation rate could be lowered by poisoning the liver in the presence of an apparent stimulation. The marked decrease of both is evident. The large differences between the heparin and the oxalate hematocrits indicate that more oxalate than was necessary to prevent coagulation was used in this experiment. Since the heparin hematocrit values for July 19th and 24th were not determined it was impossible to calculate the heparin-fibrin values for these days, and none of the latter figures are given. However, the oxalate hematocrits always differed from the heparin

hematocrits by roughly the same percentage, making the fibrin figures as given comparable but not absolute.

Dog 4. Male. Given chloroform July 19, 2:30–4:30 p.m.

SAMPLE NUMBER	TIME	MILLIMETERS SETTLED IN 1 HOUR	PLASMA FIBRIN	HEPARIN HEMATOCRIT	OXALATE HEMATOCRIT	REMARKS
	1926					
1	July 19, 2:00 p.m.	4.9	0.22	41.2	36.1	Plasma clear, pale, yellow
2	July 21, 11:00 a.m.	0.7	0.06		40.0	Drowsy; not eating. Blood slightly hemolysed
3	July 22	1.0	0.19		43.5	Plasma and urine yellow
4	July 24	1.6	0.23		37.3	Plasma straw color

The dog recovered completely.

DISCUSSION. It has been pointed out by Abderhalden (6) that in jaundice (etiology not given) the rate of sedimentation of the erythrocytes is decreased, but aside from that there are few clinical conditions in the human where unusually slow sedimentation rates have been observed. In "oligoplasmie," Biernacki (7) reports that the plasma volume is only 20 to 40 per cent and that the cells settle as slowly as in normal defibrinated blood. In carcinomatosis with metastases to the liver it has been recorded that the sedimentation rate is very slow.

In our experiments we have produced an experimental jaundice associated with liver injury due to the administration of chloroform to dogs over a prolonged period. The jaundice was indicated by large increases in the bile pigments of the urine and of the plasma. A constant decrease in the fibrinogen content of the plasma and a corresponding decrease in the rate of sedimentation occurred during the time of the progressive liver necrosis, but the values returned toward normal as recovery took place. There were no marked, constant variations in the albumin, globulin and total protein percentages, except a decrease in the last named due to the fibrinogen decrease. The plasma volume percentage of the whole blood usually showed a decrease—a slight anhydremia—with a return to normal as recovery ensued. Such a change causes slowing of the settling rate of the cells but the changes occurring here are far too great to be explained by this factor alone, as will be shown by us in a later communication.

In dog 2, the high plasma volume percentage on the last day of the experiment probably explains the fact that the sedimentation rate was more rapid on this day than on the first day of the experiment, when the plasma fibrinogen content was the same. While we believe that the fibrinogen content of the plasma largely determines the settling rate, we

know that the number of cells also plays an important rôle, other things being equal. We have no reason to think that the hydremia in this dog was the result of the liver poisoning, but prefer to explain it upon the assumption that the animal had some intercurrent infection, possibly a pneumonia, to which laboratory dogs are very susceptible, especially when they have been weakened by experimentation. An infection of this nature could scarcely explain the return of the fibrinogen to normal values unless there were also a liver recovery. This follows from the experiment on dog 3, which shows that if the liver is poisoned, even in the presence of a stimulating infection (peptone in this case), there is still a decrease in the fibrinogen of the plasma.

SUMMARY

Four dogs were poisoned by prolonged chloroform administration. The resulting liver necrosis was associated with a marked drop in the plasma fibrinogen and with a greatly reduced rate of red-cell sedimentation. These changes were parallel and apparently dependent. Relative increases in the bile pigments of the plasma and the urine as well as of the lipoids of the plasma corresponded to the condition of the liver as measured by the changes in sedimentation rate and in fibrin concentration. The albumin and globulin fractions of the plasma varied only slightly.

These experiments are an added indication of the dependence of the rate of sedimentation of the red blood cells upon the plasma fibrinogen percentage.

It is a pleasure to express our appreciation to Dr. E. C. Davidson and to Mr. C. W. Mathew for their frequent assistance during the progress of this work.

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THE EFFECT OF PHYSICAL EXERCISE ON THE GASTRIC SECRETION

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It is a common lay belief that exercise immediately after eating interferes with the digestion of food. In 1832 Bell and Roche (1) stated in the appendix to their translation of Broussais' *Treatise on Physiology*, that digestion is impaired by the exercise of the body after meals; apparently, this statement was not based on experimental data but on common observation. Beaumont (2) stated that mild exercise facilitated digestion in the stomach. Bridzius (3) reviews the more recent literature and reports the results of his own experimental work on exercise after feeding in Pavlov pouch dogs. Prior to this time few well-controlled experiments had been performed, and the results were quite contradictory. In Bridzius' own experiments Pavlov pouch dogs were fed and then exercised by being made to drag a sledge on which was a definite weight, or by simply walking the animals. He worked on two dogs and found that both walking and heavy exercise inhibit gastric secretion, and that the decrease is more marked after heavy exercise than after walking. Delhougne (4) (not quoted by Bridzius) has reported that the gastric secretion in human subjects is increased both in acidity and in amount by heavy exercise. Delhougne used fractional analysis of the stomach both with no stimulation and with stimulation by alcohol. His measurement of the exercise is apparently not even roughly quantitative; he describes it merely as "Turnen, treppensteigen, usw." He conceives of this increased secretion of acid as a mechanism by which the body may compensate for a fall in the alkali reserve and blood pH induced by exercise.

METHODS. Three Pavlov pouch dogs were used. Each weighed between 17 and 20 kilos. All three dogs were trained to run on the treadmill before the pouch operation was performed. The treadmill was motor driven, gradient and speed being variable at will. The dogs were tied to the front of the mill by a rope attached to a collar. The dogs soon learned not to pull back and would run until fatigued without placing more than a slight tension on the rope.

Tests were made on each animal about two weeks after the operation;

the first 3 or 4 tests were discarded because they usually fell somewhat below the subsequent normal determinations. The routine followed in making a determination was to collect the basal secretion at hourly intervals for 2 hours; feed a standard meal consisting of 250 grams chopped meat, 100 grams white bread, and 100 cc. milk; and then to collect the secretion at hourly intervals for the next 6 or 8 hours. When exercise was given, the dogs were started on the treadmill as soon as they had finished eating. In both normal and exercise determinations the dogs were given water ad libitum with the meal, and each hour for four hours thereafter; this was to avoid any dehydration which might result from the exercise. The animals invariably drank more on exercise days. Since water is an adequate stimulus to the gastric secretion, the animals probably would have secreted more on exercise days if the exercise had not decreased the secretion.

The work on the treadmill was the only exercise which the dogs received. In order to reduce to a minimum any adaptation to the exercise the dogs were never run on the treadmill more than twice in one week. In almost all cases, the normal determinations were made when the animal was in the room where the treadmill was running; thus the considerable amount of noise which the mill made cannot have diminished the secretion.

The exercise was measured in terms of kilogram-kilometers of work per minute; it varied for the different animals between 0.15 and 0.30 kgm.-km. per minute. They were exercised for from ½ to 3 hours. The exercise was always heavy; no attempt was made to study the effect of moderate or light exercise except in one case (dog III) where moderate exercise was employed over a long period (3 hours). In every case when the animal was removed from the treadmill it was panting heavily, often showed some muscle tremor, and did not run about voluntarily. The exercise was not carried anywhere near the point of complete exhaustion, however, for normal dogs were run on the same mill for one hour at a higher speed than was used for these pouch dogs in their ½ hour runs.

RESULTS. *Dog I*—female collie, wt. 19.0 kilos.

The results on this animal are illustrated by figure 1 where the amount of HCl in milligrams (calculated from the per cent of HCl and the cubic centimeters of juice) is plotted against time. Twelve normal determinations of 10 hours each (including a 2 hour period of basal secretion prior to food and exercise) were made; the light solid line on the graph is the average of these values. Exercise determinations were similarly made on twelve days; these may be divided into two groups, as expressed in figure 1. In the first group of 7 determinations the dog was exercised for ½ hour at a rate 3.14 kilometers per hour and a gradient of 25 per cent. The average amount of work done during this period was 7.6 kgm.-km., or 0.25 kgm.-km. per minute. The second group comprised 5 determinations, the dog being exercised for 1 hour at the same rate and gradient; he performed an average amount of work of 14.6 kgm.-km., the rate per minute being the same as in group I. It will be noted that the gastric

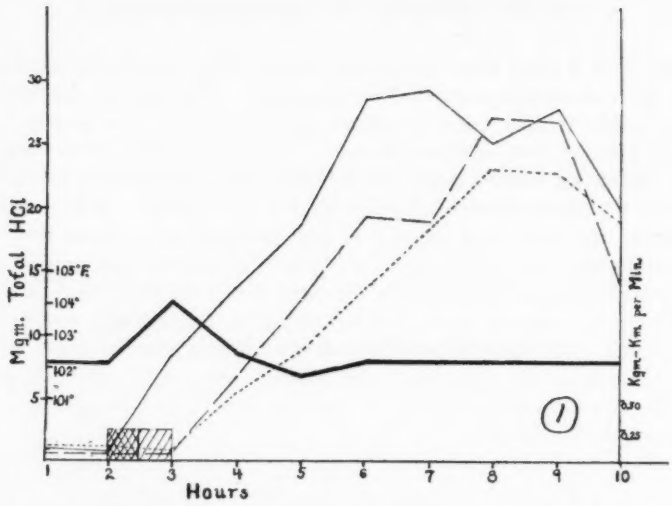

Fig. 1. Light solid line—Milligrams of HCl, average of 12 normal determinations. Light broken line—Milligrams of HCl, average of 7 exercise determinations (group I). Dotted line—Milligrams of HCl, average of 5 exercise determinations (group II). Heavy solid line—Rectal temperature for one determination in group II. Cross-hatched square—Exercise in kgm.-km. for group I. Cross-hatched and diagonally lined squares—exercise in kgm.-km. for group II.

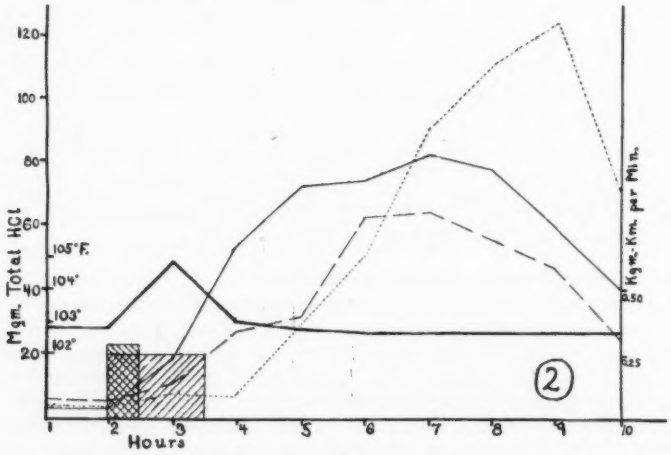

Fig. 2. Light solid line—Milligrams of HCl, average of 11 normal determinations. Light broken line—Milligrams of HCl, average of 5 exercise determinations (group I). Dotted line—Milligrams of HCl, average of 4 exercise determinations (group II). Heavy solid line—Rectal temperature, average of 3 of the exercise determinations of group I. Square with lines angling to left—Exercise in kgm.-km. for group I exercise determinations. Square with lines angling to right—exercise in kgm.-km. for group II exercise determinations.

secretion is totally depressed (does not rise above the basal level) in the hour after work; this is true both for the ½ and 1 hour exercise periods. The secretion starts to rise above the basal level in the second hour after the meal in both groups I and II, but in group II (1 hour of exercise), it rises more slowly than in group I. The rectal temperature was taken on one occasion when the dog was subjected to ½ hour of exercise, and it showed a rise of 2°F. above normal 1 hour after the meal (½ hour after discontinuing exercise).

The total HCl secretion was calculated for the entire 8 hours of each of the 24 normal and exercise determinations. The average, maximum and minimum values for the milligrams of total HCl excreted for the 8 hour period are given in table 1.

From the average figures we may calculate that after ½ hour of exercise, the total amount of HCl excreted in response to the meal was 77 per cent of the normal, while after 1 hour of exercise it was 68 per cent of the normal.

Dog II—female collie, wt. 17.0 kilos.

Figure 2 expresses the milligrams of HCl secreted plotted against time. The normal values are the averages of 11 determinations. As with dog I, the exercise determinations were divided into two groups. The first group comprises 5 deter-

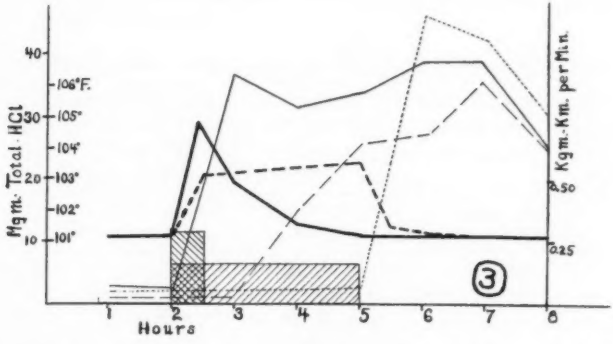

Fig. 3. Light solid line—Milligrams of HCl, average of 10 normal determinations. Light broken line—Milligrams of HCl, average of 5 exercise determinations (group I) Dotted line—Milligrams of HCl, average of 5 exercise determinations (group II). Heavy solid line—Rectal temperature, average of the 5 exercise determinations of group I. Heavy broken line—Rectal temperature, average of the 5 exercise determinations of group II. Square with lines angling to left—Exercise in kgm.-km. for group I. Square with lines angling to right—exercise in kgm.-km. for group II.

minations. On these 5 days the dog was exercised for ½ hour after eating at the rate of 0.3 kgm.-km. per minute. The second group contains 4 determinations, the exercise being given for 1½ hours at the rate of 0.25 kgm.-km. per minute. The temperature curve shown is an average of the rectal temperature readings on 3 of the days on which ½ hour of exercise was employed.

It will be noted that in this animal no complete depression of the response to the meal was obtained. With ½ hour of exercise, the secretion increases more slowly than is normally the case and never rises to the rate attained normally. Following 1½ hours of exercise the secretion is even more depressed and exceeds the basal level only very slightly during the first two hours. From the second hour on, it rises

rapidly and the maximum rate is half again as great as the normal. The peak of acid secretion comes two hours later than the normal peak. The peak of group I (½ hour of work) determinations coincides with the normal peak. The rectal temperature was taken hourly in 3 of the group I determinations; as with dog I the temperature was about 2°F. above normal 1 hour after eating and had fallen to normal 1 hour later.

The total amounts of HCl normally secreted over the 8 hour test period, that following ½ hour of exercise, and that following 1½ hours of exercise, are given in table 2. The maximum and minimum amounts secreted are also given. It will be noted that ½ hour of exercise markedly decreased the total amount secreted on the average, but that following 1½ hours of work, the increase above normal noted in

TABLE 1

	AVERAGE	MAXIMUM	MINIMUM
Normal	169.6	232.9	130.7
½ hour work	131.0	194.0	83.9
1 hour work	114.9	176.0	58.7

TABLE 2

	AVERAGE	MAXIMUM	MINIMUM
Normal	488.2	852.5	325.6
½ hour work	336.1	459.4	251.2
1½ hours work	466.2	519.4	335.2

TABLE 3

	AVERAGE	MAXIMUM	MINIMUM
Normal	211.3	263.0	166.7
½ hour work	134.3	173.3	106.6
3 hours work	130.8	146.2	123.2

the last 3 hours largely compensated for the preliminary decrease. Thus after ½ hour exercise the average amount secreted was 69 per cent of the normal, while after 1½, hours exercise it was 95.5 per cent of the normal.

Dog III—female airedale, wt. 19.5 kilos.

Figure 3 gives the average milligram total HCl excreted plotted against the time The rectal temperature taken every ½ hour is also plotted. Normally in this animal the response to the meal was rapid, the rate of secretion rising sharply and being well maintained for a period of four hours. Following ½ hour of exercise at the rate of 0.3 kgm.-km. per minute the secretion is totally depressed for the first hour after the meal and then rises rapidly in the next two hours. The secretory rate does not, following this amount of work, attain the maximum which is reached when no exercise is given. The normal values are the average of 10 determinations; the values following ½ hour exercise are the average of 5 determinations.

Five determinations were also made upon the effect of three hours of moderate exercise, the rate being 0.15 kgm.-km. per minute. With this amount of exercise the secretion is totally depressed during the exercise period of 3 hours. Upon the cessa-

tion of exercise the secretory rate rises very abruptly to a maximum which is greater than that normally reached.

The rectal temperature curve following ½ hour of heavy exercise shows that the temperature has risen to about 4.5°F. above the normal at the end of the exercise period. One hour after the meal (½ hour after cessation of exercise) it has fallen to about 1.7°F. above normal. When moderate exercise is given for 3 hours the rectal temperature rises in the first ½ hour to 2°F. above normal and remains at about that level for the entire exercise period. When the exercise is discontinued it drops abruptly to normal.

The average amounts of HCl secreted in the entire 8 hour periods normally, following ½ hour of heavy exercise, and following 3 hours of moderate exercise are shown in table 3. This table also gives the maximum and minimum amount secreted. Thus after ½ hour of exercise the total amount of HCl secreted is decreased to 63.5 per cent of the normal. After three hours work it is decreased to 61.7 per cent of the normal. That the decrease after 3 hours exercise is not appreciably greater than the decrease produced by ½ hour of exercise, even though the secretion is totally suppressed for 3 hours by 3 hours of work and for only 1 hour by ½ hour of work is due to the compensatory rise to a secretory rate above normal which takes place immediately following the 3 hour exercise period.

Discussion. The outstanding finding in all three animals is that exercise depresses the gastric secretion during the exercise period. All extrinsic factors which might tend to depress the secretion were controlled as far as possible. Thus the animals were trained to become well accustomed to the mill before the operation was performed, they were given water ad libitum in order that dehydration would not affect the result, and they were in most cases kept in the room where the treadmill was running during the normal determinations so that the only variable factor would be work.

As might be expected, there is considerable variation in the effect of the same amount of exercise in different animals. All showed a very marked depression of gastric secretion during the exercise period. With dogs I and III the secretion was depressed to the basal level; that is, the meal was not able to stimulate secretion during the period in which the dogs were at work. In dog II the stimulation by the meal during the exercise period was very slight. On the cessation of exercise the secretion always rises sharply. In dogs II and III, the rate of secretion increased above the normal level after the longer exercise periods; this is most marked in dog II. It is worthy of note that in dog II there was a greater total output of HCl in the 8 hour period after the longer exercise period than after the shorter. This is due to the fact that after the longer exercise period there was a compensating effect in the last 3 hours (the animal secreted more HCl than normally). This compensating effect is also present, though less marked, in dog III, where the total output of HCl was practically the same for both ½ hour and 3 hour exercise periods, although the 3 hour period caused a much longer primary depression.

Whether this increase above the normal in the later hours of digestion is due to an acidosis caused by exercise it is impossible to state, since no attempt was made to determine blood reaction or alkali reserve. There can be no doubt, however, that in the dog heavy exercise causes a primary depression in gastric secretion. This contradicts the work of Delhougne on man. But the only means we have of judging the severity of the exercise used by Delhougne is that he tells us that it was heavy; he made no attempt to measure even roughly the amount of work which he used. Also there can be little doubt that fractional analysis in man does not give as consistent results as can be obtained from the Pavlov pouch dogs, and Delhougne reports only one normal and one exercise determination on each of his three subjects, whereas at least 10 normal and 10 exercise determinations were made on the dogs reported here.

Heavy exercise undoubtedly causes a redistribution of the blood, more blood being sent to the muscles and probably less to the viscera. As shown by the temperature curves, the amount of exercise which these dogs were given also causes a marked rise in body temperature, the maximum rise being 4.6°F. Meyer, Cohen and Carlson (5) have shown that a rise in temperature of 2 to 4°F., whether produced by injections of foreign proteins or by external heat, is sufficient to markedly reduce the gastric secretion in dogs. Kastler (6) has shown that exercise decreases the total acid soluble and inorganic phosphorus in dogs. According to the theory of Maly phosphates enter into the reaction by which HCl is liberated. Kastler's findings also show a compensatory increase in the phosphorus to a level above the normal, which may be significant in view of the compensatory increase in gastric secretion in the later stages which occurs in dogs II and III. No attempt at an evaluation of the part played by each of these 3 factors (redistribution of blood, rise in temperature, and change in blood constituents) can be made; it is only possible to point out that any or all of them may be effective in reducing the gastric secretion when an animal is exercised.

Thus, experimentation confirms the common observation that heavy exercise interferes with digestion, and gives evidence for the statement that rest after meals is beneficial.

SUMMARY

1. In 3 Pavlov pouch dogs heavy exercise (from 0.15 to 0.3 kilogram kilometer of work per minute) for periods varying from ½ to 3 hours markedly depressed the gastric secretion during the exercise period.

2. In 2 of these dogs the secretion during exercise did not rise above the basal level; that is, the meal was not able to stimulate.

3. There was a concomitant rise of from 2 to 4°F. in rectal temperature during the exercise period.

4. In the last 2 or 3 hours of the digestive period two of the dogs showed an increase in secretory rate to a point well above the normal.

5. The probable causative factors concerned in these changes are discussed.

I wish to thank Dr. A. C. Ivy for suggesting this problem. During the performance of the work Dr. Ivy and Dr. W. J. Meek gave suggestions and criticisms which were very gratefully received.

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THE DISTRIBUTION OF ARSENIC IN THE TISSUES AFTER SERIAL ADMINISTRATION OF NEOARSPHENAMINE

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In 1922 Underhill and Davis (1) reported an investigation on the excretion of arsenic after serial administration in man of arsphenamine and neoarsphenamine. It was shown that after each injection there is a greater elimination of arsenic the second day after injection, a higher excretion, and a larger percentage of excretion, before the next injection. From these facts they suggest that after the tissues have absorbed a certain amount of the drug they take up no more and the excess arsenic is then quickly eliminated by way of the urine and feces.

According to the data which have so far appeared in the literature, the liver retains the largest quantity of arsenic of all the organs tested. Bulmer (2) states that immediately after the injection the liver contains the greatest amount, but that the quantity in the lungs soon exceeds it. The rapid lowering of arsenic content of the liver, he believes, is due to elimination through the bile.

We have attempted to determine whether the hypothesis of Underhill and Davis is subject to experimental verification by serial injections of neoarsphenamine. It would be reasonable to suppose, if a sufficient number of injections were given, and if the tissues do become saturated with the drug, that some evidence of the saturation point could be obtained. It may be stated at once that the original object of this investigation was not attained. On the other hand, data were obtained which are of considerable interest in a study of the distribution of arsenic in the tissues after neoarsphenamine introduction.

METHODS. Full-grown dogs of both sexes maintained upon a constant mixed diet received intravenously injections of neoarsphenamine by either the jugular or femoral veins. The injections were given every four days, the total number of injections varying from one to six. Six hours after the final injection the dogs were bled to death. The organs were removed, dried and analyzed for arsenic. The method of Chittenden and Donald-

[1] The data are taken from the dissertation presented in 1925 to Yale University by Alice Dimick in candidacy for the degree of Doctor of Philosophy.

TABLE 1

Distribution of arsenic in tissues after administration of neoarsphenamine

Injection neoarsphenamine intravenously

DOG	NUMBER OF INJECTIONS	WEIGHT	DOSAGE	ARSENIC PER 100 GRAMS FRESH TISSUE											
				Liver	Right kidney	Left kidney	Spleen	Heart	Thyroids	Adrenals	Muscle	Brain	Testicles	Ovaries	Bile per 100 cc.
		kgm.	*gm. per kgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5	1	10.0	0.035	0.35	0.34	0.32	0.21	0	S.P.T.	1.03	0.01	S.P.T.	S.P.T.		2.74
6	1	3.0	0.083	1.96	0.79	0.63	1.95	0.32	2.31	S.P.T.	0.05	S.P.T.		0	80 18
21	1	15.0	0.020	1.65	1.01	1.29	0.90	0.23	1.16	0.56	0	0	0.12		35.29
1	2	18.0	0.017	2.46		0.12	0	0.06	0.45	0	S.P.T.		0		
7	2	12.0	0.025	0.36	0.08		0.12		0.11	S.P.T.	S.P.T.	0	0		25.83
11	2	2.0	0.100	1.47	0.53	1.91	S.P.T.	0.19	1.44	0.78	0.21	0	0.37		24.07
17	2	6.1	0.020	0.54	1.22	1.19	0.06	0.32	0	0	0.09	0	0	0	10.45
2	3	10.0	0.020	2.05		0.46		0.59	1.36	0.35	0.18			0.55	17.18
12	3	8.0	0.021		1.12	1.19	1.00	0.32	S.P.T.	0.58	0.18			S.P.T.	31.75
10	3	14.0	0.027	0.98	3.02	1.90	1.27	S.P.T.	2.39	0.49	0.20	S.P.T.	0		117.30
18	3	18.4	0.020	1.00	1.07	1.14	1.70		S.P.T.	0.87	0.08	S.P.T.		0	3.69
9	4	11.5	0.026	0.61	0.61	0.62	0.67		0.43	0.60	0	0.01		5.10	54.39
20	4	13.4	0.020	1.52	0.91	0.85	1.01	0.07	0.20	0.72	0	0	0.06		16.02
15	5	12.0	0.025	0.58	1.41		5.43	0.07	0	S.P.T.	0.11		0.13		18.09
8	5	6.0	0.040	2.19	2.54		1.59	0.22	2.56	0.27	0.42	S.P.T.		0.63	19.55
19	5	12.6	0.020	0.87	2.94	2.04	2.31	0.24	1.48	2.17	0.05	0	0.44		19.54
16	6	15.0	0.020	0.61	1.94	2.49	2.91	0.22	0.41	1.02	0.07	S.P.T.		3.16	

S.P.T. = slightest possible trace.

son (3) was employed for digestion of the tissue and the arsenic in the final fluid was estimated by the modified Gutzeit method suggested by Sanger and Black (4).

Discussion. An inspection of the data in the table will reveal several points of interest. In the first place it must be quite obvious that the quantity of arsenic stored in the livers of the different animals bears no direct relationship to the dosage of arsenic administered or to the number of injections given. Dog 21, for example, with a dose of 0.020 gram per kilo of neoarsphenamine retained in the liver much more arsenic than did dog 5 that received a larger dose of the arsenical. Again dog 6 receiving four times the dosage of dog 21 retained in the liver only slightly more arsenic. Similar results are yielded with dogs 1 and 7 receiving two injections of arsenical. With similar doses of neoarsphenamine in four doses dogs 9 and 20 showed livers containing very dissimilar quantities of arsenic. Moreover, the livers of those dogs receiving five or six injections of the arsenical yielded less arsenic than some of the animals with a smaller number of injections. Compare dogs 16 and 21.

The kidneys show an entirely different behavior. In general although the deposition is not in direct ratio to the dosage of arsenical injected there is a distinct tendency for the quantity retained to increase significantly with the number of injections. Moreover, the relative deposition between kidneys and liver shows that more is stored in the kidney than in the liver. The spleen shows a similar behavior, but this is somewhat varied and probably is regulated by its action as a scavenger, collecting precipitates and altered red blood cells.

The thyroids and adrenals store considerable quantities of arsenic; the male reproductive organs little, the ovaries on the other hand, a considerable quantity.

The quantity in the brain is surprisingly small. It was long ago observed by Chittenden and Smith (5) that the quantities of arsenic contained in the brain vary with the nature of the compound administered. Chittenden believed that only easily soluble forms of arsenic ever penetrate the nervous tissues to any considerable degree. On the other hand, Underhill (6) demonstrated in a case of chronic arsenic poisoning from arsenious oxide relatively large quantities of arsenic in the brain and stated that at times at least "repeated ingestion of arsenious oxide may lead to the deposition of significant quantities of arsenic in the brain and spinal cord." The storage of insignificant quantities of arsenic after arsphenamine administration had been pointed out previously by Voegtlin (7), Kolls and Youmans (8), Clausen and Jeans (9), by Fordyce, Rosen and Myers (10) and Brahme (11).

These combined results indicate that organic arsenicals are not sufficiently diffusible to penetrate the nervous structures. This explains the

unsatisfactory results in the treatment of tabes and general paralysis and demonstrates that even repeated injections of arsphenamine and neoarsphenamine will not penetrate nervous tissues sufficiently to deposit enough arsenic for the destruction of parasites.

If one compares the distribution of arsenic in these experiments with neoarsphenamine with those of a chronic case (5) of arsenious oxide poisoning the results are quite different. In the arsenious oxide case of poisoning the liver is preëminently the storage place, the kidney next, then follow the spleen, cord and brain, the spleen equals the brain, the heart and muscles are at the lower end of the list. With neoarsphenamine the kidneys are preëminent, then the spleen, liver, thyroids, adrenals, heart, muscle, reproductive organs and brain.

The subordinate position of the liver may be explained perhaps by a rapid elimination through the bile. That such biliary excretion occurs is demonstrated by the relatively large quantities of arsenic found in the bladder bile. On the same basis of reasoning the kidneys should contain much less than they do, but here the factor of possible renal damage enters which may militate against rapid elimination by this path, hence storage.

It would appear in view of the diversity of quantitative results obtained in this investigation that each animal in its response to neoarsphenamine administration displays an individual capacity of retention ability. Presumably this is related to some nutritional condition as yet unknown.

CONCLUSIONS

The distribution of arsenic in the tissues after serial administration of neoarsphenamine follows a different order from that characteristic of inorganic arsenic.

The quantity of arsenic deposited in the different organs and tissues does not necessarily bear a direct relationship to the quantity of neoarsphenamine introduced nor to the number of injections given. This individual variation in the concentration of arsenic in the tissues indicates an individual difference in the capacity to absorb and excrete the drug, a difference dependent perhaps upon some unknown nutritive condition of the animal.

In confirmation of the work of others it is shown that neoarsphenamine penetrates to the nervous system with great difficulty, at no time reaching a high concentration, as indicated by the content of arsenic in this tissue after serial administration of neoarsphenamine.

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SOME FACTORS INFLUENCING THE NORMAL ISOMETRIC CONTRACTION PERIOD OF THE HUMAN HEART

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One of the difficulties experienced in the attempt to determine the length of the systole of the human heart is a lack of exact data concerning the length of the isometric contraction or pre-sphygmic period of the heart cycle, inasmuch as the ventricle begins to contract an appreciable interval before it ejects any blood into the aorta.

A large number of investigators have attacked the problem, using various methods and coming to widely different conclusions both as to the length of the period and the factors which modify it. Robinson and Draper (1) as well as Wiggers (2) give summaries of this work, and show how little conformity there is in the results thus far obtained. This diversity of results is shown, not only by the varied names given to the period, but by the lack of uniformity in defining the terms used. The designation *isometric contraction period*, as given by Wiggers, and the definition of Robinson and Draper, as *the time during which the pressure in the ventricle is rising to overcome the arterial pressure*, seem to fit the case most satisfactorily, and accordingly are accepted as the basis for the present work.

Accepting the above definition, the following theoretical considerations present themselves: There being very little active muscular tissue in any of the heart valves, the position of their respective flaps at any instant will depend upon the relative pressures of the blood in the cavities separated by them. The A-V valves will close, therefore, only when the intra-ventricular pressure exceeds that in the auricle—the "effective venous pressure" of Henderson and Barringer (3). On the other hand, the intra-ventricular pressure must exceed that in the arteries in order to open the semi-lunar valves. Consequently, at the end of diastole, the intra-ventricular pressure must be less than 50 mm. H_2O—the intra-auricular pressure—, and although the auricular systole will raise the pressure in the ventricle to a slight degree, the pressure in the ventricle at the instant it begins to contract can not be greatly in excess of this amount. During the isometric period, the ventricle has to raise its pressure from that just described to a point above the diastolic arterial pressure, which normally is not less than 75 mm. Hg, an increase of probably at least 70 mm. Hg. Inasmuch as the

pressure is further raised during the ejection phase not more than 40 mm. Hg, normally, it follows that the ventricle does relatively more work during its isometric contraction than while it is actually expelling blood, particularly as the work of the isometric contraction has to be accomplished in so short a time. For these reasons, it is at least possible that a complete understanding of the normal length of the period and of the factors which modify it might give valuable information as to the efficiency of the ventricle. Such data would have not only diagnostic but prognostic value in pathological cases, and furnish one reason for this study.

METHOD. Of the methods employed for measuring this period, the most common is the comparison of the beginning of the cardiogram with that of the carotid sphygmogram, with correction for transmission time. Robinson and Draper (1), who used this method with the most satisfactory results, freely admit the inherent inaccuracies of the method and state that in their opinion an exact measurement of the period is impossible. Wiggers and Clough (4) have suggested a method whereby the period may be measured without the necessity of correction for transmission, and their method has been followed in this work.

As a basis for their calculations, the heart sounds and the carotid sphygmogram are recorded simultaneously by the Frank segment capsules, as modified by Wiggers. This method eliminates errors due to lever-fling and friction of writing points. It has been shown by Wiggers (5) that on such a record, the beginning of the main vibrations of the first heart sound marks the closure of the A-V valves, while the primary upstroke of the carotid sphygmogram denotes the opening of the aortic semi-lunar valve, the two points which mark the boundaries of the period in question. Moreover, he further shows that the beginning of the second heart sound vibrations, which marks the actual apposition of the valve flaps, is synchronous with the bottom of the incisura of the carotid sphygmogram, thus giving a common point with which to compare the limits of the isometric contraction period, and making unnecessary the measurement of transmission time.

The procedure of Wiggers and Clough (4) is described as follows:

> . . . place a pair of dividers with one point on the onset of the first sound vibrations and the other on the second sound vibrations and transfer the set dividers to the carotid curve so that one point is placed at the bottom of the incisura. The other point then cuts the wave (at a place) representing the beginning of the isometric period. The interval between this mark and the rise of the primary wave gives the isometric period, . . .

In the work here reported the more laborious, but in our opinion more accurate method of calculating the differences mathematically, was employed. This was made possible by so arranging the timing device that

its shadows extended completely across the recording film, and by running it at double speed, successive shadows marking intervals of 1/50 second (see fig. 1).

Certain inherent difficulties arise in reading the curves. The exact foot-point of the carotid tracing is quite often uncertain. In all such cases the same rule was followed here as in the work of Lombard and the author (6) in estimating the length of the systole: ". . . . the length of the systole is the time between the point where the tracing first begins, not simply to curve, but to bend sharply upward at the foot of the primary rise of the sphygmogram . . . ." Sometimes the incisura is doubled, but such cases were discarded as unreliable. In several cases the preliminary vibrations of the first heart sound do not appear on the record, making it im-

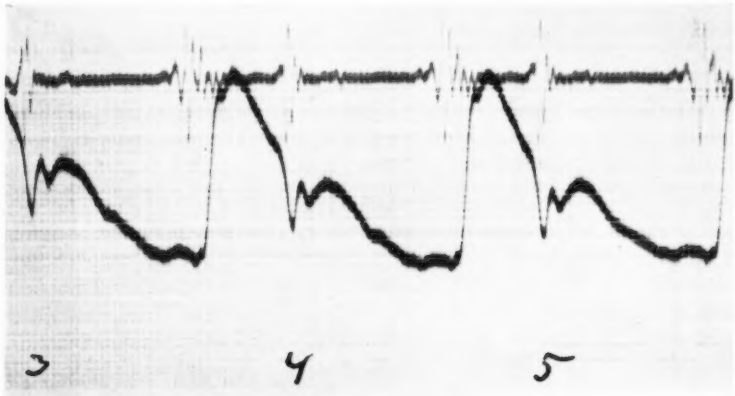

Fig. 1. Simultaneous optical tracings of heart sounds and carotid sphygmogram. Time in $\frac{1}{50}$ second. Section of curve 72–3.

possible to determine the exact beginning of the period. These also were left out of our calculations. To further insure uniformity of results, only those curves were utilized in which at least 15 consecutive cycles could be accurately read.

Because the length of systole had been found to be greatly affected by posture and the sex of the subject, records were taken from both men and women, in each of the three positions, standing, sitting, and recumbent. In all, 256 curves from 59 men and 22 women were taken, in each of the positions, but the calculations were finally made from the records of 33 men standing, 37 men sitting, and 36 men recumbent. The number of reliable curves from the women examined was not sufficiently large to be used as a basis for calculation, and consequently the consideration of the sex-factor

is left for a later communication, when more data have been obtained. This is admittedly not a large number of cases upon which to base conclusions, but it is large enough to give suggestive results, as it means the measurement of at least 1600 cycles.

Inasmuch as all calculations were made by the same individual especial care was taken to check and re-check all figures and mathematical operations as well as to plot as many curves as possible to bring out errors, and no attempt was made to make any comparisons or draw conclusions until after all the records had been read and recorded.

Having found it necessary to take the average of 15 consecutive systoles

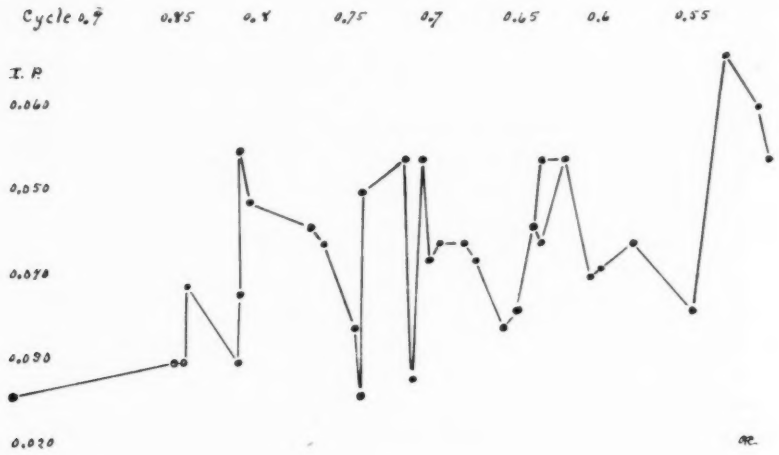

Fig. 2. Relation of cycle length to isometric contraction period in the standing position. Ordinates—isometric period in seconds; abscissae—cycle length in seconds.

to determine the average systole length for any individual, a similar number of cycles was used and their isometric periods calculated, with the expectation that some sort of a relationship might be found between them and either the systole or cycle length.

FACTORS MODIFYING THE ISOMETRIC CONTRACTION PERIOD. *1. Rate.* Influenced by the results of our previous work on the factors governing the length of systole, it was expected that, contrary to the conclusions of Robinson and Draper (1), Keyt (7) and others, the isometric period might be found to be influenced by the same factors which govern the length of systole and hence to decrease in length more or less in proportion to a decrease in cycle length. The data collected were therefore classified according to the position of the subject. All records for each class were arranged

in descending order of average cycle length, and the corresponding isometric periods were plotted as ordinates against cycle lengths as abscissae. Figure 2 is the plot made from the data of men standing. The general tendency of the curve is seen to be to rise with a decreasing cycle length, i.e., the isometric period apparently increases in an *inverse ratio* to the cycle length.

The following explanation of this unexpected result suggests itself: As the isometric contraction period marks the interval beginning at the instant that the intra-ventricular pressure becomes greater than that in the veno-auricular reservoir (and closes the A-V valves), and ceasing when the pres-

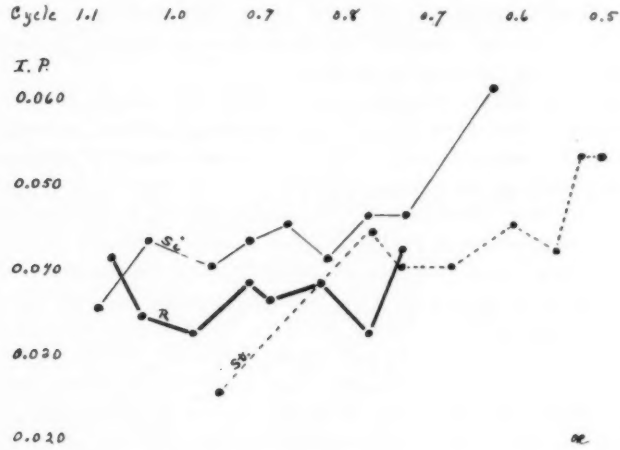

Fig. 3. Relation of cycle lengths to isometric contraction period, cycles being arranged in groups differing not more than 0.050 second in length. Ordinates—isometric periods in seconds; abscissae—cycle lengths in seconds. *St.*—standing; *Si.*—sitting; *R*—recumbent.

sure overcomes the resistance offered by the diastolic arterial pressure, (and opens the semi-lunar valves), an increase in the intra-ventricular pressure just before the ventricular contraction would decrease the amount by which the ventricle would have to raise the pressure in order to open the semi-lunar valves. Consequently, other factors being unchanged, the ventricle might be expected to accomplish this result in a shorter period of time—i.e., to shorten the isometric period. Such a condition—increase in intra-ventricular pressure just before the beginning of ventricular contraction—has been shown to exist in the long cycles in the standing position, when there is sufficient time for blood to accumulate in the veno-auricular reservoir and enter the ventricle during diastole, while, owing to the

effect of gravity, there is not time for the blood to accumulate in the reservoir and fill the ventricle during the short diastoles of rapid rates. Hence, at the beginning of ventricular contraction, the pressure in the ventricle is so low that not only relatively but actually is a longer time necessary to raise the intra-ventricular pressure above the diastolic arterial pressure.

2. Posture of body. These facts are brought out more plainly when the data for men in each position are arranged in groups of cycle lengths differing not more than 0.050 second, and plotted as before (see fig. 3).

A study of figure 3 shows that the effect of decreasing cycle length in increasing the length of the isometric contraction period is greatest in the standing position, when gravity has its greatest effect in preventing the return of venous blood during rapid rates, and least in the recumbent posture, since in that position the blood does not have to overcome the force of gravity in its return to the heart.

On the other hand, the isometric period in the sitting posture is uniformly longer than even that of the standing position, at equal cycle lengths. This seems an exception to the above-described differences between the standing and recumbent postures, and therefore requires further explanation. Such explanation is at least suggested by a consideration of the effects of changes of posture on the diastolic pressure, the resistance which the heart has to overcome before the aortic valve can open. Ellis (8) has shown that in passing from the recumbent to the head-up 45° position, (which is analogous to the sitting posture), there is an increase of diastolic pressure which is as great as the increase following the passage from the recumbent to the standing posture. Consequently the relative increase in pressure on changing from the recumbent to the sitting posture is greater than from the recumbent to the standing posture, and it is at least possible that the extra work required to overcome this diastolic pressure has more influence on the length of the isometric period that the relatively slighter effect of differences in diastolic filling of the ventricle in the two positions.

3. Strength of beat. It suggests itself that a vigorously beating heart might be able to raise its intra-ventricular pressure from the infra-venous to the supra-arterial level in less time than one which was able to contract only feebly, and such a possibility constitutes one of the theoretical uses of this period as a measure of the efficiency of the heart. Robinson and Draper (1) consider the strength of the beat as a very important factor in determining the length of the isometric period. Unfortunately the method used in this work gives no criterion by which the strength of the beat may be measured, so far as we have been able to apply it. But it is hoped that further work may enable us to obtain data concerning this important factor.

Normal variations in the length of the isometric period. From the foregoing considerations, it is evident that there can be no exact single figure for the

normal length of the isometric period. The averages found under varying conditions and the limits of the variations under those conditions are shown in table 1.

It will be seen that the isometric period varies from 0.022 to 0.068 second, with an average of 0.041 second, which is shorter than the time given by Robinson and Draper, but within the limits suggested by Wiggers.

The positive results of this study clearly indicate the necessity for further work on the subject, and we hope at an early date to be able to report

TABLE 1

Length of isometric period under different conditions

All data given in seconds

POSITION	AVERAGE CYCLE LENGTH	VARIATIONS	AVERAGE I. C. P. LENGTH	VARIATIONS
Standing	0.697	0.943–0.500	0.043	0.026–0.066
Sitting	0.813	1.086–0.628	0.044	0.030–0.068
Recumbent	0.884	1.070–0.768	0.037	0.022–0.060

upon other factors, both normal and pathological, which may modify the length of the isometric contraction period.

SUMMARY

Using the method suggested by Wiggers and Clough for measuring the isometric contraction period of the human heart, optical records were obtained from 59 men in the standing, sitting, and recumbent postures. Eliminating all doubtful records, the average isometric contraction periods of 15 consecutive cycles were obtained in 106 curves of 47 men, and the results plotted.

These results suggested that the isometric period in the standing position at least, is inversely proportional to the cycle length.

The isometric period is found to be influenced by the heart rate, the position of the body,and probably by the strength of the beat of the heart.

The effects of rate and posture are due at least in part to the diastolic filling of the ventricle and to the height of the diastolic pressure in the arteries, and hence to the difference between the infra-auricular and supra-arterial pressures at the close of ventricular diastole.

The total variations in the length of the period are found to lie between 0.022 second and 0.068 second, with an average of 0.041 second for all cases.

A more complete study of these and other possible factors modifying the length of the period is planned, to be reported later, at which time special attention will be paid to the effects of pathological circulatory conditions upon this particular portion of the heart cycle.

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THE HEAT LIBERATED BY THE BEATING HEART

Seventh Communication

A Study of the Systolic and Diastolic Temperature Rises During Anoxybiosis[1]

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A series of experiments has been carried out in which thermocardiograms have been taken of isolated hearts of terrapin (*Malaclemmys palustris*) surviving alternately in oxygen-rich and oxygen-free media. It was expected that the wave or waves in the records due to heat of oxidative origin would disappear or be lessened in size, while those independent of oxidations would remain wholly or only slightly changed during the O_2-free bath. On the other hand, it was expected that during the bath rich in O_2 all the waves would remain much as they have been observed hitherto. If, for example, the heart normally uses little or no oxygen during systole and most or nearly all of its oxygen during the remainder of the cardiac cycle, then, in the absence of oxygen, the systolic rise to temperature that I have observed (1922) would suffer little or no reduction whereas the diastolic rises of temperature observed would suffer complete or nearly complete reduction.

Method. The method of thermomyography used in these experiments has been essentially the same as that described in part first by Snyder (1922) for heart, and then more completely in the papers on skeletal muscle by Snyder and Gemmill (1925), smooth muscle by Snyder (1927) and finally on auricular (smooth) muscle by Gemmill (1927). This includes the method I adopted (1927) for the correction of the error introduced by the slight vertical displacement of the thermopile coil during a nearly isometric muscle contraction, the correction to be used in converting the observed rises of temperature into terms of heat of muscular origin.

The method for putting the heart in an anaërobic condition was varied as the experiments progressed. I first simply filled the heart chamber with Ringer's that had been previously freed of its O_2 by washing with N_2-gas;

[1] A preliminary report of this investigation was made at the Annual Meeting of the American Physiological Society held at Rochester in April, 1927.

this Ringer was then displaced by allowing N_2 to enter the chamber as the fluid was syphoned off. Later the cyanogen method of Weizsäcker (1912) was adopted; in these experiments oxygen was alternated with N_2, the Ringer bath, used to displace O_2, containing potassium (later sodium) cyanide in concentrations from m/2500 to m/3000. The Ringer used to displace the N_2 from the heart chamber was previously saturated with O_2 and this fluid in turn was displaced with O_2. The pH of the two Ringers was kept alike by adding equivalent amounts of HCl to the Ringer containing the cyanide; but the HCl was added only after the Ringer solution had been deprived of its dissolved O_2, as completely as possible, by washing with N_2-gas. All the HCN liberated by the HCl thus was kept in the bath and not lost by the washing process. This method insured a constancy in the pH index as well as in the concentrations of cyanogen and the cations Ca, K and Na. CO_2 was not controlled and was not added to either the N_2 or the O_2 gases used. The Ringer solutions were simply used to displace the one or the other of the latter gases, and to impregnate the heart muscle with, or rid it of, HCN. Before taking records the hearts were allowed to beat suspended in the heart chamber for at least half an hour in the gaseous atmosphere. The CO_2 tension in the tissues, therefore, must have been a simple function of gaseous diffusion, the relative concentrations of the non-volatile acids within the cells, in turn, probably varying inversely with the amount of oxidations.

As to the completeness of oxygen removal when an "anoxybiotic" condition was desired, it should be stated that the N_2-gas used, being supplied by a compressed air-products company, contained nearly 1 per cent of O_2, and that the gas was not passed through a reducing agent to free it of this small amount of oxygen, since the oxidative processes of muscle require a partial pressure of oxygen much greater than 1 per cent to proceed at their optimal rate. For the first object of the experiment, therefore, further removal of O_2 was not thought to be important. Nevertheless in the latter and crucial experiments where a measure of absolute anaërobic heats was desired the N_2-gas bath was initiated with a cyanogen-Ringer bath. The main idea in using the cyanogen was to impregnate the muscles thoroughly enough with that chemical so that they no longer were capable of utilizing free oxygen even though it be present, the cyanogen having its action by rendering oxidizing enzymes or catalysts inert (Geppert, 1888; Warburg, 1921, 1923). If the heat measurements in these experiments could have been made while the hearts were suspended in, or perfused with, Ringer no special precautions to insure an O_2-free medium other than addition of cyanogen need have been taken. But since the method required that the hearts be in a gaseous medium during the taking of records the use of an inert gas fairly free of O_2 was an indispensable condition. In all the experiments where cyanogen was introduced it was assumed that the cyano-

gen remained effective in the tissues, after the removal of the cyanide bath, for periods of time long enough to obtain ample records of hearts beating in the anoxidative condition. In the light of Weizsäcker's experience the times of exposure and the concentration of the cyanogen necessary to obtain this condition are not difficult to determine. But independent of the cyanogen treatment, as appeared very clearly in the results of the first experiment, the removal of O_2 from the heart by the use of N_2-gas alone, is quite sufficient to reduce greatly the heat-production of the heart during the diastolic period.

In order to subject a heart alternately to O_2-rich and O_2-free media, the following method was finally adopted. The heart chamber was provided with inlet and outlet tubes, one of which opened at the upper, the other near the lower inner part of the chamber. The cable, a light steel wire, connecting the heart inside to the muscle lever outside the heart chamber, was passed through a glass tube with fine aperture, that pierced the top of the chamber. The glass tube held a trap filled with a few drops of oil. A sleeve of soft rubber tubing on the outer mouth of the glass tube enabled one to clamp off this outlet during the change from one bath to another. The oil-trap, while thus permitting free movement of the cable connecting heart and heart-lever, prevented any considerable diffusion between the gases within and without the chamber during the course of an experiment.

The final procedure then for an experiment was as follows. After a heart preparation was mounted on the thermopile in the chamber as desired, the chamber was closed. O_2-free Ringer, to which cyanide may or may not have been added, was then sent into the chamber through the tube reaching down to the bottom of the chamber, the air was thus displaced through the outlet tube entirely. The solution was 6 to 7°C., a little warmer than finally desired, to hasten the asphyxiation. After allowing this solution to act on the heart for a few minutes it was displaced by another identical solution having a temperature of 0°C. This colder O_2-free solution was allowed to remain about 15 minutes when it was all displaced with N_2-gas, excepting about 20 cc. that were allowed to remain behind at the bottom of the chamber. The chamber having been previously cooled and put in a thermostat set for a temperature of 0 to 1°C. (see Gemmill, 1927), one had not long to wait for the thermopile to indicate temperature equilibrium between the cold and warm junctions (proximity to null point and absence, or slow rate, of galvanometer drift). The period was usually a half-hour; but often a much longer time was allowed to elapse before thermocardiograms were taken. A few trials soon taught one how long to continue the cyanide bath. I varied the time so that in some experiments the heart was still beating vigorously, in others just had begun to show cyanide intoxication, and in others again had come to a standstill, when the cyanide bath was removed.

RESULTS. As Weizsäcker found with frogs' hearts, so I found with terrapin hearts brought to a standstill with cyanogen, that upon readmitting O_2, and washing away the cyanogen with oxygenated normal Ringer solution, the hearts would gradually recover their automaticity and renew their beats with vigor. The experiments also showed that these hearts like the frog hearts could perform work and continue their automaticity for a considerable time in the absence of oxygen. In all cases where the beats stopped the waves of heat production also stopped.

It should be stated here that the graphic records of the temperature changes observed during the heart-beat (the thermocardiograms) in these experiments had the same characteristics as those reported in the paper of 1922. There are two prominent rises of temperature, one beginning early in systole and subsiding during diastole, the second beginning in diastole and continuing for some seconds after the diastole subsides, that is, on into the period of diastasis. For this reason as before with the sensitive but slower d'Arsonval galvanometer it is necessary to have the heart-beat cover a period of more than 30 seconds, better 45 seconds, or more. These two rises of temperature although they do not coincide sharply with the times of systole and diastole, will nevertheless be referred to hereafter in this paper as the "systolic" and "diastolic" rises.

In the experiment of 1/18/27 the heart was allowed first to beat in atmospheric air at *ca* 3°C. during which time the data for thermocardiograms numbers D I-3 to D II-12 in table 1 were gotten. It will be seen here that the average value of the systolic temperature-rise is about 27×10^{-5} degree C. and that of the diastolic temperature-rise is 349×10^{-5} degree C. After expelling the air from the heart chamber with Ringer-N_2-gas and then expelling the Ringer in turn with N_2-gas alone and allowing time for the small amount of O_2 remaining to be further exhausted, records were again taken upon which the data exhibited in the table from 2:35 p.m. to 4:12 p.m. (numbers D III-13 to D IV-27) are based. It will be noted that the total tension (τ) here has been doubled. This happened because the initial tension on the heart was increased. It will be noted that this increased initial tension improved the additional tension created by the heart during systole (τ_s). I shall call this additional tension the systolic extra-tension; it represents what would be called pulse-pressure if the increased tension during contraction of the heart were expressed in terms of pressure.

The important thing one observes in table 1 is that, although a few individual readings go much higher and a few a little lower, yet the average temperature rise for the systolic wave of the beats in nitrogen is of much the same magnitude as it is for the systolic waves of the beats in oxygen. The tension during the nitrogen bath was doubled; in oxygen this may be expected to double the temperature rise, and, therefore, on a standard

basis one may say the beats in nitrogen have systolic waves not less than ½ the size of those in oxygen. The diastolic waves of temperature-rise for the beats in nitrogen on the other hand at first show a much greater reduc-

TABLE 1

Experiment of January 18, 1927

Terrapin heart, split ventricle. Weight of ventricle only, 1.89 gm.; rate of beats varied from 68 seconds to 168 seconds per one beat, the pause representing by far the greater part of the cardiac cycle. The systole + diastole varied from 15 to 30 seconds in duration. Temperature, *ca* 3°C. τ = total tension developed; τ_s = the extra tension over the initial.

THERMOCARDIOGRAM NUMBER, HOUR AND TREATMENT	TOTAL MAXIMUM TENSION (τ)	SYSTOLIC "EXTRA TENSION" (τ_s)	SYSTOLIC TEMPERATURE RISE	DIASTOLIC TEMPERATURE RISE
	gm.	*gm.*	*deg. C.* $\times 10^5$	*deg. C.* $\times 10^5$
12:30 Air, DI—3	13.6	5.1	26.2	360
4	13.6	5.1	26.2	335
5	13.6	5.1	26.2	384
6	13.6	5.1	26.2	410
12:48 7	13.6	5.1	26.2	381
DII—8	14.5	5.1	26.2	339
1:15 9	14.5	5.1	27.2	331
10	14.5	5.1	31.5	308
11	14.5	5.1	31.5	326
1:30 12	14.5	5.1	21.0	319
Average, (n) = 10	14.0	5.1	26.8	349
2:35 N_2, DIII—13	27.8	8.5	30.2	33
14	27.8	8.5	30.2	33
15	25.2	7.9	23.6	51
2:55 16	25.2	7.9	23.6	50
17	25.2	8.1	43.0	82
18	25.2	8.1	43.0	124
19	25.2	8.1	35.7	105
3:35 DIV—20	28.8	6.3	27.8	129
21	28.8	6.3	27.8	133
3:55 22	27.5	6.0	23.7	81
4:00 23	27.5	6.0	27.8	101
24	27.5	6.0	22.0	95
25	27.5	5.9	21.8	109
4:12 26	28.0	8.2	25.8	123
27	28.8	7.4	25.8	123
Average, (n) = 15	27.0	7.3	29.4	

tion, to a figure $\frac{1}{10}$ of those of the beats in oxygen, but by the end of an hour these waves increase until their value is a little more than ⅓ of the rise observed for the oxygenated heart. The diastolic temperature rise suffers

clearly much more reduction by the nitrogen treatment than does the systolic rise of temperature. When expressed as ratios of the extra-tension τ_s (but this ratio is not given in table 1) the systolic heat is more nearly the same and the recovery still more widely different in both aërobic and anaërobic conditions, than when expressed in terms of total tension. This fact and the small tension developed suggests that the heart in this first experiment, but only during the oxygen treatment, was beating isotonically rather than isometrically. The results of the oxygen part therefore can not be put on a common basis of comparison with the nitrogen part in which all the beats were isometric. The heat production during diastole, while much lower during the N_2-bath, is still of considerable magnitude, due no doubt in part to the small amount of oxygen (*ca* 1 per cent) remaining present in the N_2-gas that was used.

TABLE 2

Experiment of ii/25/27

A repetition of the experiment of i/28/27. Weight of ventricle, 3.03 grams = W_m; H_{sys}/T and H_{dias}/T are systolic and diastolic heats per gram muscle, per gram tension per beat, determined from the equation $H/T = \frac{\Theta \cdot s \cdot W_m}{\tau}$, where s is the sp. ht. of the muscle and Θ the observed systolic or diastolic temperature-rise, as the case may be.

TREATMENT OF HEART	HOUR	NUMBER OF BEATS OBSERVED	DURATION OF BEAT	TOTAL TENSION τ	$\frac{H_{sys.}}{T}$	$\frac{H_{dias.}}{T}$
			seconds	*gm.*	*cal.* $\times 10^5$	*cal.* $\times 10^5$
After 2 hours in N_2 only	1:12	8–17	36	21	1.00	1.88
After 1 hour in 21 per cent O_2 (oxygen)	2:13	18–20	61	41	1.51	9.58
	2:25	21–24	65	40	1.33	11.63
	2:40	25	39	40	1.88	8.22
Average, (n) = 8			60	40	1.39	10.42
After 2 hours in N_2 (nitrogen)	4:55	41–43	76	48	0.73	4.64

In the experiment of February 25, 1927, thermocardiograms were taken only after the heart had been beating in N_2-gas for more than two hours at 1 to 2°C. Atmospheric air was made to displace the N_2 by the Ringer route, and after one hour of recovery a set of aërobic records was taken. The heart was then treated with another N_2-bath for only one hour when a second set of anaërobic records was taken. The chief results appear in table 2.

Upon admitting atmospheric air the heart slowly recovers its heat production, faster for the diastolic than for the systolic phases. The slowness is due in large part doubtless to the low temperature at which the heart

was kept. At the end of one hour cardiac temperature-rises may be considered nearly normal again; the diastolic heat has risen to a figure equal to the highest of the aërobic hearts of the 1921 experiments (table 6). Then upon beating in anaërobiosis for two hours both systolic and diastolic heat waves have been reduced roughly to one-half of the highest values that appeared during the aëration period. The low temperature of the heart muscle again accounts for the slowness of these effects. The experiment here was discontinued.

It is clear that some rapider and more efficient method must be used to supply and withdraw oxygen from the heart muscle. In the next experiments to be reported I used pure oxygen instead of atmospheric air for the oxybiotic treatment and initiated the anoxybiotic treatment with

TABLE 3

Experment of May 20, 1927

Showing effect of cyanogen on heat production of the surviving terrapin ventricle. Weight of ventricle, W_m = 2.92 grams. Temperature, 2.8°–3.0°C. Calculation of heat ratios same as in table 2.

TREATMENT OF HEART	HOUR	ORDINAL NUMBER OF BEATS OBSERVED	AVERAGE TIME OF ONE BEAT	MAXIMUM TENSION TOTAL (τ)	$\frac{H_{sys.}}{T}$	$\frac{H_{dias.}}{T}$
			seconds	*grams*	*cal.* $\times 10^5$	*cal.* $\times 10^5$
In pure O_2, no previous N_2 or CN (cyanoge (n)	11:47	1– 6	72	33	6.07	5.87
	12:08	7–10	72	35	5.31	5.35
Average, (n) = 10					5.78	5.64
CN m/3000 for 8 minutes then	12:55	11–17	68	34	4.85	0.58
N_2 gas for 33 minutes, and	1:20	18–23	66	36	4.90	0.44
continued		24	63	35	5.81	1.17
		25	63	36	5.55	1.02
Until	1:40	26–32	60	33	5.58	0.79
Average, (n) = 22					5.01	0.61

Ringer's containing suitable concentrations of cyanogen. To determine these suitable concentrations, and other desirable procedures, three preliminary experiments were required. The results of these need not here be reported, and I pass at once to the experiment of May 20, 1927, whose results are brought together in table 3.

Comparing the heat ratios it will be seen that after oxygen was withdrawn from the heart the tensions remain throughout practically the same, and that the systolic heat (H_{sys}/T) diminishes hardly at all, whereas the diastolic heat (H_{dias}/T) drops from 5.6×10^{-5} to 0.6×10^{-5} calorie. The systolic heat dropped about $\frac{1}{8}$, the diastolic heat, however, about $\frac{9}{10}$ from the original values.

In table 4 are shown the chief results of two experiments, those of June 10 and June 16, 1927. In both cases the hearts first were exposed to the cyanogen-N_2 treatment as in the last experiment. As the figures show this treatment reduced the diastolic heat to an average of 0.79×10^{-5} calorie for the one heart and to an average of 1.02×10^{-5} calorie for the other heart, while the systolic heats were reduced to averages of 2.59×10^{-5} and 1.58×10^{-5} calorie respectively. Upon washing away the cyanogen

TABLE 4

Two experiments, using cyanogen

DATE OF EXPERIMENT	HOUR	THERMO-CARDIOGRAM NUMBER (*V*)	TREATMENT OF HEART	TIME OF WHOLE HEART CYCLE	MAXIMUM TENSION, TOTAL τ	TEMPERATURE RISES IN °C. $\times 10^5$		HEAT PRODUCTION* IN CALORIES $\times 10^5$	
						$\theta_{sys.}$	$\theta_{dias.}$	$\frac{H_{sys.}}{T}$	$\frac{H_{dias.}}{T}$
				seconds	*grams*				
vi/10/27	2:06	19–24	CN, N_2	48	43.1	33.7	7.5	2.56	0.55
	2:16	25–27	CN, N_2	52	41.8	33.9	15.7	2.69	1.22
Average, (n) = 9			CN, N_2	49	42.6	33.7	10.5	2.59	0.79
	6:44	38	O_2	216	36.5	78.3	173.0	7.00	15.40
	7:00	39	O_2	216	38.1	80.5	158.0	6.88	13.50
	7:30	40–44	O_2	202	39.3	97.6	175.0	8.10	14.40
Average, (n) = 7			O_2	206	38.9	92.4	172.0	7.75	14.40
vi/16/27	1:10	6–10	CN, N_2	30	33.9	12.0	15.0	1.44	1.26
	1:22	11–15	CN, N_2	31	26.7	15.2	12.1	1.65	1.30
	1:32	16–22	CN, N_2	31	26.7	15.2	5.9	1.65	0.63
Average, (n) = 17			CN, N_2	31	28.8	14.2	10.4	1.58	1.02
	5:33	33–40	O_2	36	30.4	44.9	67.7	4.28	6.46
	5:45	41–50	O_2	36	30.1	50.4	70.0	4.81	6.70
Average, (n) = 18			O_2	36	30.2	48.9	69.0	4.56	6.40

* For calculations see legend to table 2. Weight of ventricles, W_m = 3.95 and 3.51 grams respectively.

with normal oxygenated Ringer and exposing the hearts to pure oxygen the diastolic heat rose steadily until in the one case it was more than 18 times, and in the second case more than six times the average figure obtaining during anaërobiosis. The systolic heat in both hearts increased just about three times.

It will be noted that the mechanical tensions exerted by the hearts in all three of these experiments, where cyanogen was used, remained the same very nearly in passing from the cyanogen-N_2 to the O_2 treatment; yet the

heat production, notably the diastolic heat production, underwent profound changes.

DISCUSSION. In order to compare the results of the several experiments, their average values have been brought together in table 5. In this table are included the number of thermocardiograms (n) analyzed, the mean duration of the heart-beat, the total tension (τ) and the weight of the ventricle in each case. These factors all have some relative importance in evaluating the heat ratios shown in the last three columns and therefore require further comment.

TABLE 5

Summary showing the heat production during systole and diastole of hearts of Malaclemmys pelustris during ozy- and anozybiosis

DATE OF EXPERIMENT	TREATMENT OF HEART	(n)	DURATION OF BEAT	AVERAGE MAXIMUM TENSION (τ)	WEIGHT OF MUSCLE (W_m)	$H_{sys.}/T$	$H_{dias.}/T$
			seconds	*grams*	*grams*	*cal.* $\times 10^4$	*cal.* $\times 10^5$
i/18/27	O_2, 21 per cent	10	80	14	1.89	2.93	38.9
	N_2 only, 2 hours	15	120	27	1.89	1.68	6.00
ii/25/27	N_2, 2 hours	10	36	21	3.03	1.00	1.88
	O_2, 21 per cent, 1 hour	8	60	40	3.03	1.39	10.42
	N_2 2 hours	3	76	48	3.03	0.73	4.64
v/20/27	O_2 pure, $\frac{3}{4}$ hour	10	72	34	2.92	5.78	5.64
	CN, N_2, $1\frac{1}{2}$ hour	22	66	34	2.92	5.01	0.61
vi/10/27	CN, N_2, $1\frac{1}{2}$ hour	8	49	43	3.95	2.56	0.79
	O_2 pure, $3\frac{1}{2}$ hours	7	206	40	3.95	7.75	14.40
vi/16/27	CN, N_2, $2\frac{1}{2}$ hours	17	31	29	3.51	1.58	1.02
	O_2 pure, $3\frac{1}{2}$ hours	18	36	30	3.51	4.56	6.40

a. In the case of the heart of ii/25/27 where the average of only 3 observations are given, there were really many preceding and succeeding beats that were read directly on the galvanometer scale, that were exact counterparts of what appears in the table, but unfortunately only a few of them were successfully recorded graphically.

b. The duration of the whole heart beat affects "the duration of the muscle action," to a certain extent (a discussion of which term appears in my paper of 1927, p. 737 ff.). For, in the slowest beats where there is a definite "pause" the duration of the muscle action must be taken as some-

thing less than the whole of "the cardiac cycle,"—the period of systole, say, plus the whole of the period of diastole. If the beat is prolonged only by the lowered temperature of the heart chamber, the muscle action and duration of whole cycle have some regular proportionality, but if the prolonged beat of the ventricle is due to block in conduction then the pause doubtless no longer has the same proportionality to the rest of its cycle, as it has in beats where there is no block.

c. The results of the first experiment, so far as the beats in oxygen are concerned, are due largely as already stated to the low initial mechanical tension (and therefore probably to the isotonic character of those beats,—the very high value for H_{dias}/T in the O_2 part surely suggests a Fenn-effect) and ought not to be included in this comparative table. But the mechanical tension during the N_2-bath was nearly doubled and thus the latter beats were as fully isometric as in any other experiment; these therefore may be added for the purpose of comparison. In all the other experiments the beats were as isometric as one may make a heart muscle contract isometrically and still maintain its automaticity. Let us now proceed to a discussion of the main points the experiments bring out.

1. Can the waves of temperature-rise, that I have heretofore observed during systole and diastole, be mere artifacts? If these waves were due to an alleged slipping over junctions of the thermopile, or to a failure to stir the media surrounding the heart or the heart chamber; or to an alleged gross difference of temperature between the lower and upper strata of the media filling the heart chamber, then surely simply exchanging this medium from atmospheric air, or from oxygen gas to nitrogen gas, all at the same atmospheric pressures, and the same temperature levels with the heart remaining undisturbed in the heart chamber attached to the same lever, the thermopile undisturbed, often the tensions (both initial and total) remaining exactly the same,—surely under these circumstances the alleged defective mechanical devices and defective temperature control would still be operating precisely in the same ways and to the same degree, and therefore according to my critics the temperature waves during systole and diastole should continue just the same experiencing no marked increase or decrease of any regularity whatever. But instead of this placid indifference to the nature of the gas surrounding the heart we see our temperature waves, as registered by the thermopile for each cardiac cycle, being anything but indifferent. In the experiment of ii/25/27 the mechanical tensions of the heart muscle are nearly the same at two points, one in atmospheric air, the other in N_2; yet the heat waves instead of remaining stationary rise with the increase of O_2 tension and fall with its decrease.

Nor does the period of time allowed to elapse, after the Ringer's bath, change this effect. In the experiment of v/20/27 at the end of $\frac{3}{4}$ of an

hour in the O_2-bath, the heat ratios are 5.78 and 5.64; upon changing by the Ringer route to the cyanogen-N_2-bath, and allowing $\frac{1}{2}$ hour to elapse, the heat ratios are respectively 5.01 and 0.61, the latter figure the diastolic heat. One and one-half hours should have been time enough for the N_2-gas to have settled down into strata of very different temperatures, time enough to produce the same heat waves found in the O_2-bath. But inspection of table 3 will convince one that we are dealing here with a difference to be ascribed only to differences in metabolism of the heart in the presence and absence of oxygen, and not to artifacts.

I conclude, therefore, that these experiments give a final rigorous proof showing that the temperature waves, that I observed occurring during systole and diastole of the spontaneously beating terrapin ventricle (1922), are due to intrinsic temperature changes within the heart muscle, and are to be dealt with as seriously and with as much confidence in their importance as any of the well-known cardiographic or electrocardiographic waves, or as the measurements made upon that material that has been in many ways so satisfactory for myothermal studies, namely, the skeletal muscles of the frog.

2. But granted that these waves in the thermocardiograms are measures of actual flare-ups of heat during the heart-beat one asks, why do they vary so much in their magnitudes for different hearts even when their conditions and their mechanical performances are much the same? For example in the last three experiments (table 5) the H_{syt}/T values for the three different hearts when in pure oxygen may be as low as 4.56 or as high as 7.75, and the H_{dias}/T ratios may be as low as 5.6 or as high as 14.4. In answer it may be pointed out that the chemical conditions for no two hearts were ever really the same. In one case the heart had not yet been subjected to CN or intentionally to any other condition leading to anoxybiosis, in the second case the O_2-gas succeeding CN and N_2 had continued for $1\frac{1}{2}$ hours and in the third case for $2\frac{1}{2}$ hours before the records were taken. The high values of H_{dias}/T for the heart of the first experiment during oxybiosis are still more striking. But this already has been explained in part as a heart beating not isometrically; but part of this high value I believe is also due to a previous (unintentional) asphyxiation, occurring probably during the period of isolating and mounting the heart on the thermopile. It may be here pointed out that the preparation of the heart was always carried out at room temperature, furthermore that the operation involved a sudden cessation of the normal process of aëration provided by the animal itself. In this case the vigorously beating organ doubtless rapidly accumulates fatigue products, that are gradually burned in the oxygen bath of the cooled heart-chamber while at the same time the products of the surviving beats are taken care of. This may explain in part the very high H_{dias}/T value found in the first experiment. The high value in the second experiment

(ii/25/27) seems to have no other explanation than this, for here the heart was first purposely asphyxiated with N_2, and the beats were all fully isometric. In the case of v/20/27 if asphyxiation occurred during preparation, the recovery in pure oxygen was sufficient to oxidize most of the accumulated fatigue products, before records were taken. In the experiment of vi/10/27 the O_2 period directly follows one of severe asphyxiation; the H_{diss}/T value again is very high. The cyanogen treatment and asphyxiation of the heart in the experiment of vi/16/27 was the severest of all. Three and one-half hours in oxygen shows an H_{diss}/T value of only medium magnitude.

It should be also pointed out that the experiments were done at very different seasons of the year, and that the hearts used were those of cold-blooded animals.

Again, as in the present series of experiments, the hearts have been subjected not only to various tensions of oxygen, but to a treatment that profoundly affects the oxidases, or catalysts of the tissues. If cyanogen attaches itself to the catalyst either by adsorption or in loose chemical union or not, in any case one can not be sure upon washing away the cyanogen and readmitting oxygen, when the reversal of the cyanogen effect is complete. The behavior of the heat production at least would indicate that the complete recovery of the full mechanical action of the heart muscle is not in itself an index of such complete reversal of the cyanogen effect. Finally, one must admit the possibility of unknown intrinsic uncontrolled factors that, like cyanogen, may retard, or contrariwise, accelerate oxidations and, therefore, heat production.

In view of all these considerations there can be little surprise that variations appear among the heat ratios under the most uniform conditions of the experiments.

This lack of uniformity in the condition of the various hearts makes it impossible to say what the normal or balanced oxybiotic values of the systolic and diastolic heat ratios may have been.

By the term "balanced oxybiotic heat ratios" I mean those ideal values for the heat ratios where the changes, physical and chemical, within the heart are complete for each heart beat, leaving no residue of products or actions, belonging either to the so-called initial or recovery periods of the muscle action, to be disposed of during succeeding heart beats.

3. In search for such ideal oxybiotic heat ratios I have reviewed the results of the experiments that formed the basis of my 1922 paper. The results of these earlier experiments, when reduced to the same standard heat ratios as has been done above with the results of the present experiments, appear in table 6.

In considering the heat ratios of these older experiments it must be pointed out that there are two factors that detract from their accuracy.

First, the results could not be corrected for the slight vertical displacement of the thermopile coil, a correction that has been applied to all the results of the present investigation. The absence of this correction tends to leave the two ratios 5 and 10 per cent too high. Second, the temperature of the hearts in those earlier experiments was higher than that of the hearts of the present experiments, being from 7.5 to 12.5°C. in the former as against 0 to 3°C. in the latter. The higher temperature tends to cut short the time for the readings of the systolic temperature-rises and, therefore, tends to make them somewhat too low.

4. Keeping in mind now the limitations to be put upon the results of both the older and the present experiments we may proceed to compare them as summarized in tables 5 and 6.

TABLE 6

*Summary of average results of experiments on hearts beating in atmospheric air**

DATE OF EXPERIMENT 1921	(n)	W_m	τ_i	τ	SYSTOLIC TEMPERATURE RISE	DIASTOLIC TEMPERATURE RISE	$\frac{H_{sys.}}{T}$	$\frac{H_{dias.}}{T}$
	beats	*grams*	*grams*	*grams*	*deg. C.* $\times 10^5$	*deg. C.* $\times 10^5$	*cal.* $\times 10^5$	*cal.* $\times 10^5$
February 26.	8	1.4.	14	28.8	18	57	0.7	2.3
May 4.....		2.8	16	25.3	12	110	1.1	10.0
May 25.....	5	2.0	19	28.0	66	91	3.9	5.4
June 4.....	11	2.7	16	26.8	45	93	3.7	7.9
June 5.....	5	3.7	24	38.0	79	135	6.3	10.8
June 10 a...	5	1.5	36	45.0	19	65	0.5	1.8
June 10 b...	3	1.5	8	20.0	16	239	[1.0]	[14.8]
Mean of (n) = 7 hearts.....							2.7	6.4

* Data for this table and the heat ratios will be found for the most part in table 2, p. 269 of my 1922 paper, those for June 4 being combined. I bracket the ratios for the b part of the June 10 experiment, for the initial tension (τ_i) indicates an isotonic rather than isometric contraction. The H_{sys}/T and H_{dias}/T ratios are calculated from the equation for H/T as given for table 2.

a. The variations in the heat ratios among the 1921 hearts (all beating in 21 per cent oxygen) while large, are less widely divergent than they are among the 1927 hearts. This accords with the least that one could expect, since the object in the former was uniformity of treatment throughout.

b. Comparing the 1921 ratios (all oxybiotic) with the anoxybiotic and oxybiotic ratios of the 1927 hearts, one gets the impression that even among the oxybiotics there must have been prevailing very different degrees of chemical activity, especially oxidations, at different times and for different hearts, although the mechanical performances were often much the same. There must have been at times inhibitions or retardations of the heat producing processes, at other times accelerations and augmentations of these

processes, and all out of proportion to the mechanical performances of the heart muscle. Taken beat by beat, as far as the heat production among these isolated hearts goes, the all or none principle does not seem to hold.

5. Directing attention again to the newer results as shown in table 5 one may point out that

a. Low oxygen tension alone does not directly lessen to the utmost the processes leading to heat production, mechanical response remaining the same. But an initial treatment with cyanogen (table 5, v/20/27) followed with low oxygen tension (N_2-gas) brings the diastolic heat near to the vanishing point, leaving the initial heat only slightly reduced. This accords well with our notions concerning free oxygen entrapped within the tissues, the nature of the action of cyanogen itself, and the anoxidative processes going on in skeletal muscle during the period of rising mechanical tension.

b. Low oxygen tension, and more promptly cyanogen followed by low oxygen tension, if prolonged, also reduces the systolic heat ratio, although the mechanical tensions remain the same. But this reduction of the systolic heat does not proceed nearly to the same extent as does the reduction in the diastolic heat. This suggests that some oxidation occurs during the systolic period, if oxygen is present, and possibly an inhibition or retardation of the exothermal physico-chemical processes belonging to the "initial" period of the muscle action.

c. The most remarkable result of the investigation appears in the last two experiments (table 6) where pure oxygen was admitted to the hearts after they had been treated with cyanogen and allowed to beat a long time in oxygen-want. During the anoxybiosis thus imposed the systolic heat production is of a magnitude well within the range for oxybiotic hearts, being 2.56 and 1.58 respectively, but the diastolic heat production is reduced to figures far below any observed for the oxybiotic condition. Now upon admitting oxygen both the systolic and diastolic heats increase until the former has become three times greater for both hearts, and the latter has become 18 times greater for one heart and more than 6 times greater for the second heart. What should be emphasized (and the experiment of ii/25/27 should be also here considered) is the fact that during oxybiosis which follows a period of anoxybiosis the heat production of both phases of the heart beat not only increases but tends to increase far beyond the average values found for hearts not previously subjected to oxygen-want. The great rebound in the increase of the heat ratios after asphyxiation is ended appears in the experiment of ii/25/27 to a very marked degree. The fact that this occurs without exception where oxygen-plenty follows oxygen-want would seem to put the phenomenon outside the category of mere chance, while those cases of high diastolic heat production observed with no certainly known preceding oxygen-want (i/18/27

table 5, and v/4/21 and vi/5/21, table 6) come under a high suspicion of having previously suffered, by some unknown agent or agents, a generous dose of anoxybiosis.

This phenomenon suggests that the "recovery" process goes on in the resuscitating heart during the act of contraction (systole) as well as during the act of relaxation (diastole). To determine what the true anoxybiotic and oxybiotic heat production may be in each of these two mechanical phases of the muscle action will now require some further analysis.

6. In the discussion thus far, and in the presentation of my results, I have spoken of the heat produced during the contractile act of the heart muscle as systolic heat and that produced during the relaxation of the muscle as diastolic heat. The question that may now be asked is, to what extent does the systolic phase of heat production of the ventricular muscle correspond to the "anoxidative-initial phase," and to what extent does the diastolic phase correspond to the "oxidative-recovery phase" of heat production as we understand these terms as used in the Hill-Meyerhof statement of the processes involved in contraction of frog's skeletal muscles?

From what we have seen it is clear that the "pure anoxidative" heat of the heart muscle is to be had from the determinations on the hearts beating in anoxybiosis, and that if these anoxidative quantities are subtracted from the corresponding quantities observed for hearts beating in oxybiosis, then we have, in the remainders, values that may be termed "pure oxidative." The shortness of the systolic period, I believe, does not give time for an appreciable heat of oxidative origin to arise when the hearts are in N_2-gas alone, therefore the results of the 2nd experiment may be included in the systolic part of this analysis; but those of the first experiment, because the heart was beating in part isotonically, must be omitted from the analysis altogether. The analysis for the systolic period then is as follows:

Mean anoxidative and oxidative, systolic heat production in the terrapin's ventricle

	HEART NUMBER				MEAN
	2	3	4	5	
Total systolic h.p. during oxybiosis, following anoxybiosis	1.4	5.8	7.7	4.5	4.85
Total systolic h.p., during anoxybiosis (pure anoxidative initial h.p.)	0.7	5.0	2.5	1.6	2.45
Pure oxidative, initial h.p., following directly upon anoxybiosis	0.7	0.8	5.2	2.9	2.40

From this analysis it appears that the initial heat ratio of pure anoxidative origin may be as low as 7 or as high as 50 microcalories (1 microcalorie =

1×10^{-6} cal.), with a mean of 24.5 microcalories; on the other hand the portion of the initial heat that is of pure oxidative origin may be as low as 7 or as high as 52, with a mean of 24.0 microcalories. In other words, one-half of the systolic heat of a heart in oxybiosis (directly following anoxybiosis) may be of an oxidative origin and one-half of an anoxidative origin. The mean total oxybiotic initial heat of such hearts turns out to be 48 microcalories, as against 27 microcalories for the heart that had been subjected to no special anoxybiosis (see mean value of H_{sys}/T, table 6).

It would seem that the oxygen-hungry heart, whenever its oxygen supply again becomes plentiful, utilizes the systolic as well as the diastolic period for ridding itself of accumulated fatigue products (or for "paying its oxygen debt" as A. V. Hill puts it for skeletal muscle).

In evaluating the heats of the diastolic period we now may not use the results also of the second experiment for the pure anoxybiotic values, for the diastolic period is so long that the small amount of O_2 in the N_2-bath may have added heat of oxidative origin. Confining our consideration then to the results of the three cyanogen hearts, the analysis is as follows:

Anoxidative and oxidative heats (directly following anoxybiosis) during the period of diastole of the terrapin's ventricle

	EXPERIMENT NUMBER			MEAN
	3	4	5	
Total diastolic h.p. during oxybiosis (total diastolic h.p.)	5.6	14.4	6.4	8.8
Diastolic h.p. during anoxybiosis (pure anoxidative diastolic h.p.)	0.6	0.8	1.0	0.8
Pure oxidative diastolic h.p.	5.0	13.6	5.0	8.0

The portion of diastolic heat that we may call pure oxidative diastolic (recovery) heat thus may be as low as 50 or as high as 136, or have a mean of 80 microcalories; the portion of the total diastolic heat of oxybiotic hearts that is purely anoxidative is only about $\frac{1}{11}$, being only 8 out of a total of 88 microcalories. This mean total diastolic heat here again is higher than the mean H_{dias}/T for the 1921 hearts, the latter being but 64 microcalories (table 6); and here again it appears that the oxygen-hungry cyanogen hearts during their newly-gained oxybiosis are utilizing the time of diastole even more than that of systole to recover not only from each preceding systole but from the accumulated arrears of former beats in anoxybiosis.

The sum of both total systolic and total diastolic heat gives a figure for the whole heart beat (oxidative plus anoxidative in both diastole and sys-

tole) of 8.8 + 4.5, or 13.3 $\times$ 10^{-5} calorie, a figure a good deal larger than the sum of the "average" heats of the 1921 series (table 6), this latter being 2.7 + 6.4, or 9.1 $\times$ 10^{-5} calorie and a figure itself that may be somewhat higher than the true value.

It is obvious now that during systole or the initial period of the ventricular beat, if oxygen is present in sufficient amount heat of oxidative origin is liberated. Since the beat and the mechanical tension developed by the contracting muscle go on for a time just as well in the absence of oxygen we need not count this systolic heat of oxidative origin as a part of the processes immediately concerned with the act of muscle contraction,—we need not consider it a part of the "initial" heat to use the term now current in works on skeletal muscle physiology. Indeed in the light of this latter work it appears most probable that this systolic oxidative heat really belongs to the "recovery" heat of the previous muscle action.

7. What now may be said of the proportions between initial anoxidative and recovery oxidative heats, between total anoxidative and total oxidative heats for a single beat of "normal" ventricular muscle? Our analysis in the last section gave a mean value of 2.4 $\times$ 10^{-5} calorie for the initial anoxidative heat. We may take this as a mean representative of one and one only twitch, but the mean value found for the recovery oxidative (8 $\times$ 10^{-5} cal.) is too high, including as it probably must oxidation of some of the accumulated fatigue products as well as that of the preceding systole. The mean heat ratios for the aërobic hearts of the 1921 experiments (table 6) are doubtless freer from this source of error, and in this respect lie nearer to the true values for oxybiosis. If one may introduce a correction for the vertical displacement of the thermophile coil these 1921 ratios surely would be the truer values. This factor of coil-correction for the 1927 series has averaged about 4 per cent for the systolic temperature rises and 8 per cent for the diastolic temperature rises. In the 1921 series the temperature of heart chamber was higher, this cut the contraction period shorter, the coil displacement, however, was slightly greater. If a reduction of $7\frac{1}{2}$ per cent be made from the mean systolic and 15 per cent from the mean diastolic values (table 6) the correction is probably more than actual calibrations would have demanded; but the error in any case will be much less. With this correction the systolic and diastolic heats for the aërobic 1921 series of hearts, instead of being 2.7 and 6.4, become 2.5 and 5.4 respectively.

Using these corrected mean heat ratios of the 1921 series for hearts beating in oxybiosis, and the mean values gotten in the above analyses of the 1927 series for hearts beating in anoxybiosis, the following list may be made for comparison. The figures express absolute calories $\times$ 10^5 per gram ventricle muscle, per gram tension exerted, per spontaneous beat:

Systolic heats,—total initial, 1921 hearts	2.5	
pure anoxidative, initial	2.4	
pure oxidative, initial		0.1
Diastolic heats,—total delayed, 1921 hearts	5.4	
pure anoxidative, delayed	0.8	
pure oxidative, delayed		4.6
Total oxidative, initial + delayed, heat		4.7
Total anoxidative, initial + delayed, heat		3.2
Total initial + delayed, anoxidative plus oxidative, or total systolic + diastolic, heat		7.9

8. The absolute values in this list when converted into their simplest ratios finally are of interest when compared with the original analysis of this kind made by Hartree and Hill (1922, p. 377) from their results on the heat production of the sartorius muscle of the frog. These authors record no initial oxidative heat production for the frog's muscle, and I think the small value shown above for the ventricle may be considered a quantity well within the experimental error; from which one may safely conclude that even in oxybiosis a well aërated heart muscle produces a negligible amount of oxidative heat during systole. On the other hand, as shown in table 5, an oxygen-hungry, cooled heart upon exhibition suddenly to a sufficient oxygen supply will produce a marked quantity of heat of oxidative origin during the (initial) systolic period. The long systolic period of the cold ventricle, lasting from 5 to 8 seconds, gives time for such oxidation whereas the very short initial contraction period of the frog's sartorius at 20°C. lasting say from 0.05 to 0.08 second gives practically no time even in oxybiosis for oxidation.

On the other hand the delayed (diastolic) anoxidative heat of the ventricle is relatively large compared with the larger initial (systolic) anoxidative heat, being 0.8:2.4 or 1:3.0. This ratio of anoxidative heats for the two phases of muscle action is nearly the same as Hartree and Hill found for frogs' sartorius, namely, 0.3:1, or 1:3.3. Delayed anoxidative heat therefore must be considered as a characteristic and important feature of terrapin ventricle as it is of frog sartorius muscle; the cause or causes of this heat may be the same in both muscles.

In the ventricle the initial anoxidative heat is nearly one-half as much as the delayed oxidative, the ratio being 1:1.9; this ratio for frog sartorius is 1:1.2; the ratios of total initial to total delayed heats in the two kinds of muscle are 1:2.16 and 1:1.5 respectively.

It thus appears that the delayed heat in terrapin ventricle is still relatively a good deal more than it is in frog sartorius, although much nearer

to it than found in my earlier (1922) study of the subject. Indeed the present analyses of the heats for ventricle oblige me to abandon the precise thermochemical origin that I assigned to them at that time; and now rather than attempt to fit the present data in with the physical and chemical events that have been in part demonstrated, and in part hypothecated, for skeletal muscle, I prefer to await further facts, more indubitable evidence, of what really happens in the various stages of the whole muscle action.

9. While thus the quantitative distribution of the heat production within the muscle action has not as yet been shown to be identical for different kinds of muscle, it is of importance to emphasize that

a, the mean total heat produced during the whole of a muscle action (when the muscle responds to a normal intrinsic stimulus in an isometric contraction), as shown above for cardiac cross-striped muscle (7.9×10^{-5} cal.), is very nearly the same figure as Snyder (1927) found as the mean total heat produced by a purely smooth muscle organ, the terrapin's cardia (gastric) sphincter, namely, 7.7×10^{-5} calorie;

b, the mean total initial heat for the cardiac muscle (2.5×10^{-5} calorie) likewise is only 11 per cent less than the mean total initial heat found by Snyder and Gemmill (1925) for the frog's gastrocnemius (in oxybiosis) in the isometric response to a single stimulus sent in through its nerve, namely, 2.8×10^{-5} calorie. If this figure bears the same ratio to the total heat of gastrocnemius under the same conditions as does total initial to total heat as shown above for ventricle (2.5:7.9 or 1:3.16) then the mean total for gastrocnemius would by 8.8×10^{-5} calorie, a figure that is 11 per cent greater than that for ventricle and again within the experimental error;

c, the mean total heat for one of the two experiments with Lumulus heart beating spontaneously, (also a cross-striped cardiac muscle, and likewise contracting against an isometric lever) is also very near the mean total heat production of terrapin ventricle, namely 7.07×10^{-5}. (Snyder, 1926, p. 174).

SUMMARY

1. Using methods previously described the heat waves produced by the spontaneously beating ventricle of terrapin (*Malaclemmys palustris*) have been recorded with the organ surviving in anoxybiosis, the latter condition being produced by reducing the oxygen partial pressure to nearly one per cent, and later by treating the heart with cyanogen.

2. In the anoxybiotic condition thus established the terrapin ventricle still responding to the normal sino-auricular stimulus continued to beat for several hours exerting maximum tensions quite equal to those produced during oxybiosis, during which time, although the mechanical performance of the heart muscle and all conditions of the experiment remained the same, excepting the presence of oxygen, the thermocardiogram waves exhibited

a profound alteration. This phenomenon is taken as rigorous proof that the systolic and diastolic waves of temperature-rise previously described by the author (1922) were in no sense artifacts but entirely reliable indices of waves of heat-production accompanying the progress of intrinsic physical and chemical events that are involved in the heart muscle during its normal beat.

3. During the anoxybiosis initiated with cyanogen, it was assumed that the heat of oxidative origin was reduced to zero, but nevertheless heats of considerable magnitudes appeared both in the systolic (initial) and the diastolic-post-diastolic (delayed) periods of the ventricular beat. These heats it was concluded were of purely anoxidative origin.

4. When reduced to terms of a common standard of measure, namely, calories of heat per beat per gram ventricle muscle and per gram tension exerted (in isometric contractions) the heats of purely anoxidative origin while varying considerably give mean values of 2.4×10^{-5} calorie for the systolic (initial) phase and 0.8×10^{-5} calorie for the diastolic (delayed) phase of the muscle action. The ratio of these two heats for ventricle muscle is nearly the same, namely, 1:0.3, as was found by Hartree and Hill for the anoxidative (initial and delayed) for sartorius muscle of the frog. But a further comparison of ratios of the various phases of heat production shows that they differ considerably for the two different muscles. It is concluded, therefore, that the earlier attempt to correlate the heat production in ventricular muscle with the known physical and chemical events, and with the newer hypotheses that concern skeletal muscle must be abandoned for the present.

5. Both systolic and diastolic waves of heat production increased greatly upon changing a heart from the anoxybiotic to the oxybiotic conditions, the increase being much the greater in the diastolic wave. The total heat production for the whole cardiac cycle in this case was much greater than was found for hearts not previously exposed to anoxybiosis. It is concluded, therefore, that the oxygen-hungry heart muscle *a*, utilizes oxygen during the systolic as well as the diastolic phase of its beat, and *b*, during each beat also oxidizes not only materials liberated in the particular beat that is in progress, but also a moiety of the accumulated, unburned products of preceding beats that had been carried out with insufficient oxygen supply.

6. Upon comparing the heat production in anoxybiosis with the values obtained from hearts beating in oxygen and that had been not previously subjected to anoxybiosis the mean values of the waves of heat production, (when reduced to the same standard terms as given in 4 of this summary) show *a*, that the heats of purely oxidative origin are 0.1×10^{-5} calorie for the systolic (initial) phase, and 4.6×10^{-5} calorie for the diastolic (delayed) phase of the muscle action; *b*, that the combined heat of purely

anoxidative and oxidative origins has a mean value of *ca* 2.5×10^{-5} calorie for the total systolic (initial) phase and 5.4×10^{-5} calorie for the diastolic (delayed) phase, and 7.9×10^{-5} calorie for the total heat production of the whole muscle action.

7. The mean values of heat production for the various phases and for the whole of a heart action compare favorably with such as have been determined for other kinds of muscle when responding isometrically to a normal intrinsic stimulus.

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ADDENDUM

During the reading of the final proof of the above paper the report of Prof. H. Bohnenkamp's communication to the German Physiological Society, on the heat-production of the frog's heart during O_2-want and cyanide treatment, has come to my attention (Ber. d. gesammt. Physiol., current number). Professor Bohnenkamp speaks of his experiments as being the first of the kind, obviously not knowing of the preliminary report of these experiments on the terrapin heart (This Journal, lxxxi, 509). In any case the frog's heart cannot be made to beat as slowly as the terrapin's heart; it therefore will be impossible to separate the initial (systolic) from the recovery (diastolic) waves of temperature rise for that heart, as I have done for the terrapin heart, and thus to show the marked difference oxygen-want produced in the magnitude of these two principal sources of heat accompanying a muscle-action.

THE INFLUENCE OF YEAST ON THE ALIMENTARY RATE[1]

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Herbromez, quoted by Murlin and Mattil, recommended yeast as a purgative; Cevrey, quoted by the same authors, used yeast for the suppression of diarrhea in infants; and Faissons and Roos, mentioned in the same article, report yeast as a corrective for various alimentary disorders. Brewers' yeast was used in making these observations. Murlin and Mattil (1) report a series of experiments on the influence of yeast on gastro-intestinal motility. In their tests the number of experimental subjects is small and the length of the experiment is short. They report varying results, some of their subjects finding an increase in the number of stools whereas others were constipated. All taking raw yeast reported gas production objectionable. The bulk of the stools was increased, indicating either an increased water content or decrease in the efficiency of digestion. They conclude that yeast has a laxative effect. Daniels (2) reports marked diarrhea in infants when yeast is used as the source of vitamine B. Davison (3) reports a similar finding, and concludes that yeast is of no benefit in chronic intestinal indigestion. Diarrhea and loss of weight made it injurious in infant feeding. Hawk (4) and his co-workers have reported cases in which relief was secured from chronic constipation and gain in body weight by the use of bakers' yeast. Smith (5) reports a mild laxative effect followed by some constipation after the use of rather large quantities of yeast. Hence, it is apparent that the balance of evidence favors some laxative action of yeast, either by the CO_2 production, high vitamine B content, or change in the intestinal flora.

EXPERIMENTAL METHODS. Two phases of the problem were taken up in this work: 1, the effect of yeast on the rate of passage of food residues through the intestine; 2, the effect of the yeast on the number of bowel movements daily.

Rats were used in the first phase, because of the nature of their diet, the convenience in using them and the vast amount of nutritional work being done on them. Both albino and hooded rats were used (young adults). Small colored glass beads were used to measure the intestinal

[1] This investigation was aided by a grant from the Fleischmann Co.

rate. Dr. Ellen Stewart, working in this laboratory, has checked this method against various others and finds it efficient. It is favorably recommended by Alvarez and Friedlander (8) in work on man, and Elliott and Barclay-Smith (9) have used it successfully. Dyes were rejected because the initial appearance and final appearance are difficult to determine. The smallest size glass beads were used and bright colors were chosen.

The animals were fed twenty beads in a small gelatine capsule, the time of the initial appearance of the beads in the feces was determined and the feces collected at regular intervals thereafter until all the beads were recovered. The animals were maintained on an adequate diet which served

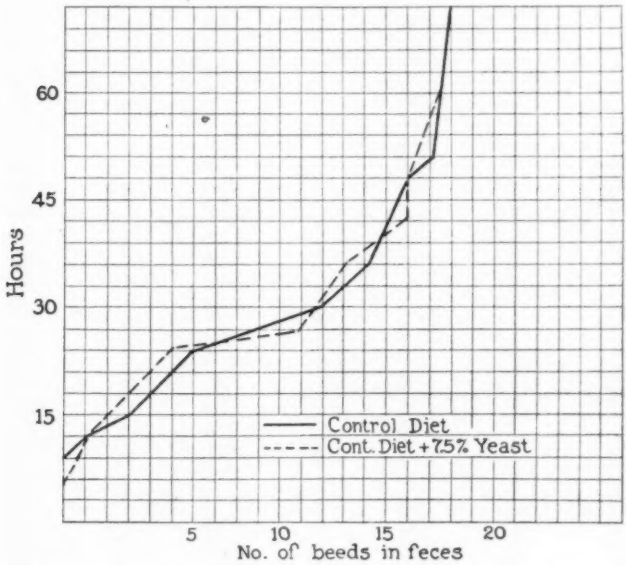

Fig. 1. Adult rats. Average rate of elimination of small glass beads in the feces on standard diet (43 tests) and on standard diet + 7.5 per cent yeast (43 tests).

as the control. The control diet consisted in the main of bread, milk, corn, sunflower seeds and raw carrots. The initial appearance and total digestion time were determined by the method outlined above. The animals were then fed the same ration to which was added seven and one-half per cent by weight of fresh bakers' yeast (Fleischmann). The same determinations were then made on this yeast diet. The averages for forty-three observations on the control diet and an equal number on the yeast diet (7.5 per cent yeast) are shown in figure 1.

In addition to the 7½ per cent yeast diet, varying amounts, up to 25 per cent of the diet, were used. Furthermore a series of determinations were made in which bone ash in the amount of 10 per cent was added to the diet. Neither of these alterations produced any significant variation in the intestinal rate.

TABLE 1

Human adults on diets selected by each individual

SUBJECT NUMBER	AVERAGE NUMBER OF BOWEL MOVEMENTS PER DAY		
	First control period (16 days)	Yeast period (16 days)	Second control period (16 days)
1	1.56	1.69	1.22
2	2.00	2.06	1.83
3	1.50	1.71	2.14
4	1.00	1.47	1.09
5	1.37	1.53	1.30
6	1.17	1.41	1.08
7	1.93	2.46	2.16
8	1.57	1.61	1.66
9	1.53	1.55	1.80
10	1.20	1.35	1.00
11	1.17	1.28	0.81
12	1.00	1.07	1.12
13	1.57	1.28	1.36
14	1.53	1.71	1.78
15	1.68	1.71	1.53
16	1.00	1.33	1.35
17	1.85	1.83	2.16
18	1.80	1.64	1.42
19	1.28	1.60	1.31
20	1.58	3.46	1.71
21	1.31	1.11	1.07
22	1.62	2.07	1.75
23	1.06	1.56	0.92
24	1.33	1.35	1.28
25	1.00	1.00	1.25
26	1.61	1.53	1.93
27	2.00	1.71	
28	1.00	1.17	
29	1.31	1.28	
30	0.87	0.92	1.00
31	2.25	1.69	2.14
Average.....	1.44	1.58	1.47

In the second phase of the work volunteers (students and instructors) in physiology were used, during the summer and autumn, 1926. Sixty volunteers started the experiment during the summer. These individuals

TABLE 2

Human adults on diets as selected by each individual

SUBJECT NUMBER	AVERAGE NUMBER OF BOWEL MOVEMENTS PER DAY		
	First control period (16 days)	Yeast period (30 days)	Second control period (16 days)
1	2.00	2.46	2.29
2	1.33	1.37	1.71
3	1.13	2.42	1.20
4	1.46	2.21	1.78
5	2.85	2.17	2.18
6	2.80	2.67	2.40
7	2.22	2.17	2.00
8	2.14	2.14	1.78
9	1.13	1.14	1.07
10	1.07	1.67	0.80
11	2.00	2.00	2.00
12	1.20	1.31	1.28
13	1.57	1.37	1.42
14	2.12	2.78	2.14
15	0.57	1.03	0.92
16	1.91	2.10	1.81
17	2.00	2.42	2.14
18	1.71	1.79	1.60
19	1.36	1.59	1.28
20	2.13	2.14	2.40
21	1.31	1.53	1.28
22	3.53	3.55	3.07
23	1.00	1.92	1.93
24	0.86	0.82	1.21
25	2.64	1.92	2.00
26	1.42	1.48	1.38
27	1.80	1.66	1.71
28	1.46	1.31	1.35
29	1.28	1.27	1.13
30	0.78	1.20	1.00
31	1.40	1.77	1.56
32	2.28	1.91	1.50
33	1.28	2.30	2.00
34	1.06	1.00	0.92
35	0.71	0.92	1.00
36	1.07	1.04	0.93
37	1.40	1.26	1.23
38	1.35	1.07	0.92
39	1.33	1.20	1.15
40	0.92	1.13	1.06
41	1.85	1.99	1.56
42	0.54	0.28	0.43
43	2.35	2.35	2.00
44	2.71	3.67	3.00

TABLE 2—*Concluded*

SUBJECT NUMBER	AVERAGE NUMBER OF BOWEL MOVEMENTS PER DAY		
	First control period (16 days)	Yeast period (30 days)	Second control period (16 days)
45	2.00	1.96	1.90
46	1.06	1.03	0.92
47	1.18	0.85	1.20
48	2.42	2.53	2.84
49	2.00	2.70	2.13
50	2.00	3.13	1.64
51	2.71	2.23	2.58
Average.....	1.66	1.80	1.62

recorded the number of bowel movements per day for a period of two weeks, continuing their usual diets and habits. After this period they added three cakes of yeast (Fleischmann's small cubes) per day to their diet for a period of two weeks, again keeping record of the number of stools per day. At the termination of this yeast period another two weeks without yeast was recorded. The results of this experiment are summarized in table 1.

In the autumn eighty-nine people volunteered and the experiment was conducted along the same lines except that the yeast period was of four weeks' duration instead of two, thus making the whole period of observation eight weeks instead of six. The results of this experiment are summarized in table 2. Only about 60 per cent of the subjects completed the experiment. Some of the failures were due to the fact that yeast was disagreeable to eat in the necessary amount, as well as to gastro-intestinal discomfort from gas formation.

The results on rats indicate that there is no variation in the alimentary rate in normal rats upon a diet containing varying amounts of yeast up to 25 per cent. However, no variation was observed in a series of similar observations when a high percentage of bone ash was incorporated in the diet. Theoretically this should have a constipating effect on rats as it does on other animals, notably dogs. The inference from these bone ash determinations is that the alimentary motor mechanism in rats is not easily modified by diet.

It has been constantly noticed in the animals used throughout these experiments that the form and gross consistency of the feces remained unchanged regardless of the variations in diet. A possible exception to this will be noted later. Never in any of the yeast periods was there a consistent tendency toward loose stools in the rats.

The first series of experiments on man shows an interesting tendency,

as analyzed in table 3. That is, those individuals who normally have a sluggish bowel, as indicated by a low number of movements daily show a more marked increase than do those whose bowels are more active than the average, the increase in the former being 14 per cent while the average in the lattter is 0. The increase for this whole group is 8.6 per cent. In fourteen of the thirty-one individuals of experiment L, there seem to be more positive effects. Of these fourteen, eight fall in the group whose normal was below the average; whereas, of the seventeen who seem to

TABLE 3

Analysis of tables 1 and 2 on the basis of intestinal rates below and above the general average

	CONTROL PERIOD (16 DAYS)			YEAST PERIOD*			CONTROL PERIOD (16 DAYS)			CHANGE DURING YEAST PERIOD
	Low	High	Average	Low	High	Average	Low	High	Average	
Experiment I										
										per cent
A. All subjects (31)	0.87	2.0	1.44	1.0	3.46	1.58	1.0	2.0	1.47	+8
B. Subjects having intestinal rates below average (15)	0.47	1.33	1.14	0.93	1.56	1.30	0.81	1.30	1.12	+14
C. Subjects having intestinal rates above average (15)	1.56	2.00	1.73	1.28	3.46	1.75	1.23	2.16	1.77	0
Experiment II										
A. All subjects (51)	0.54	3.53	1.66	0.28	3.67	1.80	0.43	3.07	1.62	+10
B. Subjects having intestinal rates below average (26)	0.54	1.51	1.17	0.28	2.42	1.34	0.43	1.93	1.21	+12.6
C. Subjects having intestinal rates above average (25)	1.28	3.53	2.22	1.66	3.67	2.36	1.64	3.07	2.10	+9.2

* Experiment I = 16 days, experiment II = 30 days.

be unaffected only seven fall in this group, the remaining ten being in the group whose normal is above the average. All of which seems to indicate that yeast has a slightly greater influence in individuals with a sluggish bowel.

In the second series as analyzed in table 3, twenty-three of the fifty-one appear to be slightly affected. This is practically the same proportion as in experiment I, and of these twenty-three twelve were in the more sluggish group, the remaining eleven falling in the more active group. In the twenty-eight who seem to have been unaffected fifteen were in the more sluggish group and thirteen in the active. This does not support the indications shown in the first series. There is practically no difference

in the per cent of increase in the two groups of the second experiment, the per cent in the sluggish group being 12.6 and in the active group being 9.2, while the increase for the whole series is only 10 per cent.

There is some difference in the ages of the people in the two experiments, the first series being taken during the summer when the average age of the students is a little higher than during the regular autumn quarter.

These individuals were requested to note any unusual subjective sensations during the period of yeast ingestion. The only reports which were at all constant were, 1, an over-production of gas both in the stomach and in the bowel, which in some cases became quite distressing, and 2, a tendency to looser stools during the yeast period. This latter observation fits into the findings of Hawk, and Murlin and Mattil, that the per cent of water in the stools was increased during the yeast period. Some observations were made on rats covering this point. The fresh feces were weighed, dried to constant weight and the percentage of moisture determined. By this method the percentage of moisture was found to be approximately 8 per cent higher during the yeast period. This determination was made using yeast which had been dried at room temperature for forty-eight hours.

The softening of the feces by this additional moisture content might contribute to a feeling of well-being in spite of the conclusions of Burnett (14) that the slow rate and well-formed fecal units probably accompany unusual good health.

The high content of vitamine B in yeast might be expected to result in some action since Gross (15) has reported that lack of vitamine B results in a very slow intestinal rate.

The people in the second group made reports of their weight during the eight-week period but no consistent variation was found.

No clear-cut conclusions seem to be warranted on the basis of the experiments on man. In the first place, observations on the alimentary rate of so many persons covering six to eight weeks permits uncontrollable variables to enter, even though no case of actual illness is included in the series. It was hoped that these variables would be neutralized both by the number of the subjects and the duration of the experiments. In the second place, while subjects 7 and 20 (table 1), and nos. 3, 4, 10, 15, 23, 33, 49, 50 (table 2) seem to show a significant average increase in alimentary rate, other individuals show an average, though less distinct, decrease. It is difficult to accept these opposite results as really due to the one factor, yeast, since the apparently delayed rate was not confined to individuals with diarrhea or showing a pronounced distaste for yeast. In the latter case the tendency to slowing of the intestinal rate could be accounted for by nervous inhibition. We wish to call particular attention to subject 42 (table 2). This was a vigorous and healthy medical student, despite

the marked constipation, according to the common standards. There was nothing unusual about his diet. The slow intestinal rate was not a temporary condition, but had been in evidence for at least ten years prior to this experiment. The subject did not have the headaches and depression that frequently accompany constipation. During the four weeks of yeast eating the intestinal rate was reduced 100 per cent, the average being one bowel movement every fourth day. This subject showed neither liking nor disliking of the yeast.

CONCLUSIONS

1. Yeast in varying amounts up to 25 per cent of the total diet has no effect on the alimentary rate in normal rats on an adequate diet.

2. In prolonged tests on eighty-five "normal" human adults on usual diets, approximately 55 per cent show an increase in number of stools daily during a period of yeast ingestion, but the average from the normal is very slight. And since some individuals (even "constipated" ones) may show a decreased alimentary rate on yeast, the value of yeast as a laxative in adults on average normal diets is questionable.

3. In rats there is some increase in moisture content of the stools during the period of yeast ingestion. And in man yeast has a tendency to produce softer stools either by increase in water content or increase in occluded gas.

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THE INFLUENCE OF YEAST ON GASTRIC SECRETION (MAN, DOG)[1]

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The effect of the proprietary vitamine-B preparation, Orypan, upon the secretions of the pancreas, liver and salivary glands, was investigated by Uhlmann (1). He concluded that vitamine-B stimulated the activity of these organs. Cowgill and Mendel (2), using a concentrate of vitamine-B prepared from brewers' yeast and solutions containing vitamine-B obtained from wheat embryo, rice polishings and navy bean, were unable to confirm Uhlmann's findings. The effect upon gastric secretion of adding a substance rich in vitamine-B to a test meal was studied by Gompertz and Cohen (3). Their control test meal consisted of one shredded wheat biscuit and 200 cc. of boiled water. To test the effect of yeast, 10 grams of Vegtex were added to this meal. Their results show a much greater gastric response to the meal containing yeast and they attribute to vitamine-B a succagogue action.

The evidence concerning the influence of vitamine-B preparations upon digestive secretions thus appears quite conflicting. Since the number of experiments carried out by Gompertz and Cohen seemed to us too limited to warrant drawing definite conclusions, we have attempted further investigation of this question. Experiments were designed to test the effect of bakers' yeast on gastric secretion in man and in dogs.

The individuals taking the test meals were all in good health and experienced little or no difficulty in carrying out the routine of the experiment. They came to the laboratory at their usual breakfast hour, swallowed a modified Rehfuss tube, and withdrew the residuum from the stomach. A suspension of 20 grams of yeast in 400 cc. of water was then taken. Specimens of gastric contents were withdrawn every 15 minutes during the ensuing 75 minute period. As a control test breakfast, 5 grams of powdered soda crackers were taken on alternating days suspended in a like quantity of water. The quantity of dry soda crackers taken thus equalled roughly the dry weight of 20 grams of fresh yeast. All specimens were filtered through paper filters and 1 cc. of the filtrate was titrated against N/40 NaOH. Dimethyl amino-azo-benzene and phenolphthalein

[1] This investigation was aided by a grant from the Fleischmann Company.

were used as indicators of free and total acidity, respectively. The results are expressed in percentage of HCl. Any experiments that might be questioned because of not obtaining a specimen at the proper time on account of obstruction of the tube or because nausea was felt by the individual, were thrown out of the series.

Dogs with Pavlov pouches were used for another series of experiments. The animals were in good condition and responded well to both gastrin

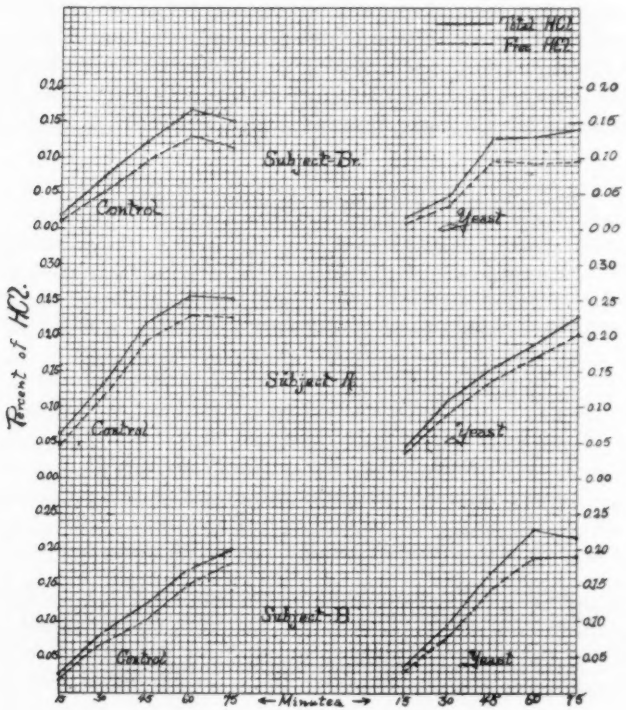

Fig. 1

and a meal of ground meat. On the day of the experiment, without having other food, they were given by stomach tube 20 grams of fresh yeast suspended in 100 cc. of water. Alternating with the yeast, a synthetic control meal was given suspended in a like quantity of water. It consisted of 5 grams of a mixture of protein (casein—52.4 per cent), carbohydrate (starch—37.1 per cent), fat (olive oil—1.7 per cent), and ash ($MgCO_3$—8.7 per cent). Dry bakers' yeast was found by Hawk, Smith and Holder

(4) to contain the above amounts of protein, carbohydrate, fat and ash. The secretion from the pouch was collected during the succeeding hour and the amount recorded. A sample was titrated, as given above, for free and total acidity.

RESULTS ON HUMAN SUBJECTS. Figure 1 shows curves of the gastric responses in per cent HCl of each of the three subjects to both the yeast and the control meals. Each curve represents the average results of eight experiments. The highest acidity for the control series in subjects A and Br was reached at the end of one hour; the highest in the yeast series, at the end of one hour and fifteen minutes. The percentage of free and of total HCl in these two subjects rose higher with the control meal than with the yeast. Subject B showed a higher acidity with yeast than with the soda crackers, while the high point was reached at the end of one hour with

TABLE 1

The effect of the test meals on the gastric secretion of dogs

ANIMAL NUMBER	TEST MEAL	NUMBER OF EXPERIMENTS	AVERAGE AMOUNT OF GASTRIC JUICE OBTAINED AT THE END OF EXPERIMENT	AVERAGE PER CENT HCl	
				Free	Total
			cc.		
1	Synthetic	7	3.49	0.220	0.297
	Yeast Meal	7	1.98	0.169	0.235
2	Synthetic	5	3.38	0.212	0.310
	Yeast Meal	5	2.36	0.164	0.235
3	Synthetic	5	3.48	0.241	0.334
	Yeast Meal	5	1.66	0.149	0.219

the yeast and not until an hour and fifteen minutes with the soda crackers. This may have been due to the fact that the latter subject rather enjoyed the flavor of the yeast preparation and disliked the cracker suspension. The other subjects did not appreciate the yeast any more than they did the control meal. The higher maximum acidity for the control meal, shown in the curves of A and Br, may be accounted for in part at least, as will be shown below, by the fact that when the two test meals were incubated in 0.191 per cent or approximately 0.2 per cent HCl at 38°C. for one hour and fifteen minutes, the acidity of the cracker suspension was found to be 0.179 per cent and of the yeast, 0.155 per cent.

RESULTS OF THE EXPERIMENTS ON DOGS. The amount of gastric juice secreted in response to the synthetic meal was invariably greater than that provoked by the yeast. The averages of free and total HCl for the control and yeast experiments are given in table 1. From the above table,

it will be seen that the percentages of total HCl of the control series in each animal are higher than that of the yeast. In animal 1, the acid-percentage of the synthetic meal was 0.062 greater than that of the yeast meal, while in animals 2 and 3 the increase was 0.075 and 0.115 respectively.

Discussion of results. Since the differences between the maximum percentages of the hydrochloric acid content (total) of the gastric samples of the cracker and yeast test meals in subjects A and Br are in close agreement with that of the in vitro tests, it seems safe to assume that the reactions, occurring in the two series, are of a similar chemical nature. The cracker suspension of the in vitro tests contained a higher percentage of HCl than did the yeast suspension. The same was true in subjects A and Br. The differences between the acid-percentages of the two suspensions or test meals were 0.024 for the in vitro series, 0.027 for subject A, and 0.026 for Br.

When the percentages of acidity of the cracker and yeast preparations, after incubation, are compared with the original percentages of acidity of the respective preparations, it will be seen that there was a diminution in the acid content in both series. In the former, the average decrease was 0.012 and in the latter, 0.036. In the case of the control tests, the decrease in acidity was in all probability due to neutralization effected by the sodium carbonate of the crackers, while in the yeast tests, it may have been the result of the neutralizing action of some alkaline salts present in the yeast and of carbon dioxide, produced by the action of the cells on the carbohydrate content of the yeast product. That carbon dioxide was the chief factor in causing the diminution in the acid content of the yeast series of experiments was demonstrated by other in vitro tests on yeast. These were performed in exactly the same manner, as previously indicated, with the exception that the yeast had been boiled vigorously for one hour and cooled before it was incubated with the acid. The average percentage of HCl of the boiled yeast tests was 0.182. The decrease in acidity was 0.010 or about the same as that of the soda crackers. It does not seem likely that the protein composition of the yeast plays any significant rôle in the disappearance of the acid, since the combined-acids of the normal yeast and of the boiled yeast tests are practically identical. The average percentage of combined-HCl in the former was 0.026 and in the latter, 0.025. When the decreases in acidity of the crackers and yeast are compared, it will be noted that the latter is 0.024 greater than the former or is the same as the difference between the acid-percentages of the cracker and yeast suspensions.

The experiments on man agree with those on dogs in that the total-acidity of the controls is higher than that of the yeast. It is much more marked in the animal series. Since the gastric samples in the dog experiments were obtained from the pouch, they consisted of pure gastric juice

which secretion was effected by the stimulation resulting from the ingestion of the preparations (yeast or control meal). In the human series, the samples were not pure gastric juice, nor were the controls of the two groups the same in composition. Consequently, experiments were performed on subjects A and Br in which the synthetic mixture (5 grams in 400 cc. of water) was ingested. The maximum percentages of the total-acidity in both subjects (0.264 for A and 0.173 for Br) was slightly higher than that of the soda crackers and was reached at the end of one hour and fifteen minutes. The in vitro test for the synthetic mixture, performed in the same manner as in the other in vitro tests, showed that the percentage of HCl neutralized by the magnesium carbonate in the mixture was 0.062. If, then, this percentage of HCl is added to the maximum percentages of HCl (total) in the synthetic meal tests of subjects A and Br and if 0.036 (amount neutralized by yeast) is added to the maximum percentages of total-acid of the yeast series in the same subjects, the percentage of HCl of the synthetic meal is 0.062 greater than that of the yeast in subject A and 0.058 greater in subject Br. These results are in close agreement with those of the dog experiments, especially dogs 1 and 2; the average of these animals was 0.068 and of subjects A and Br, 0.060. If the same computations and comparisons are made in regard to the crackers and yeast meals of the human experiments, the percentage of total-acid of the former, in the case of subject A, will be found to be 0.003 greater than of the latter and in Br, 0.002 greater. These results are within the limits of experimental error. It may, thus, be inferred that there is no difference between the gastric response to the two meals. The higher acidity in the synthetic meal is probably due to the differance in protein composition.

SUMMARY

Under the conditions set forth in these experiments, bakers' yeast did not exert a gastric secretagogue action as great as that brought about by soda crackers and water or by a synthetic test meal, consisting of similar amounts of protein carbohydrate and fat as found in yeast.

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GLUCOLYSIS IN BLOOD SAMPLES

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Before the Prudential Laboratory could offer blood analyses to its policyholders it was imperative to find some means of sending containers in which samples could be transported from distant points without significant deterioration; since there was no known way of doing this a systematic search for a method was undertaken. At first sheep's blood was treated in a large number of ways both physical and chemical which might prevent changes in the sugar content; later a more restricted study was made with human blood samples. As the work progressed the problem narrowed itself to a study of the effect of halogen hydrocarbons on glucolysis and our efforts were directed to keeping the sugar content from changing over a period of at least ten days.

The sugar of sheep's blood decreases rapidly, falling practically to zero within three days when measured by alkaline picrate solution.[1] There is a significant loss in glucose values even in the time required to bring samples from the local abattoir to the laboratory, hence in the later tests of the series the blood was treated before it left the slaughter-house.

Substance after substance added to the blood had no influence on the sugar content or else rendered the sample physically unfit. It was apparent early in this study that the problem of blood preservation primarily involves the autolytic mechanism of the blood in vitro and bacteriostatic agents as such were no more effective than those which do not materially check bacterial growth. It was found that brombenzene was exceptional in that it checked the loss of glucose. Thereupon other halogen compounds were investigated, including trichlorethylene and chlor derivatives of methane. The former was effective but the latter were not. The double linkage common to ethylene and benzene suggested the trial of unsaturated

[1] The Benedict picric acid method, modified by V. C. Myers and C. V. Bailey (Journ. Biol. Chem., 1916, xxiv, 146) was employed throughout this project for measuring the blood sugar, and the reduction of picrate is assumed to be entirely due to glucose. When the work on blood preservatives was initiated this was the method best adapted to many simultaneous determinations on small samples. It was later compared with more recent procedures, using the same kind of material, and the results assured us that other standard methods would lead to the same conclusions.

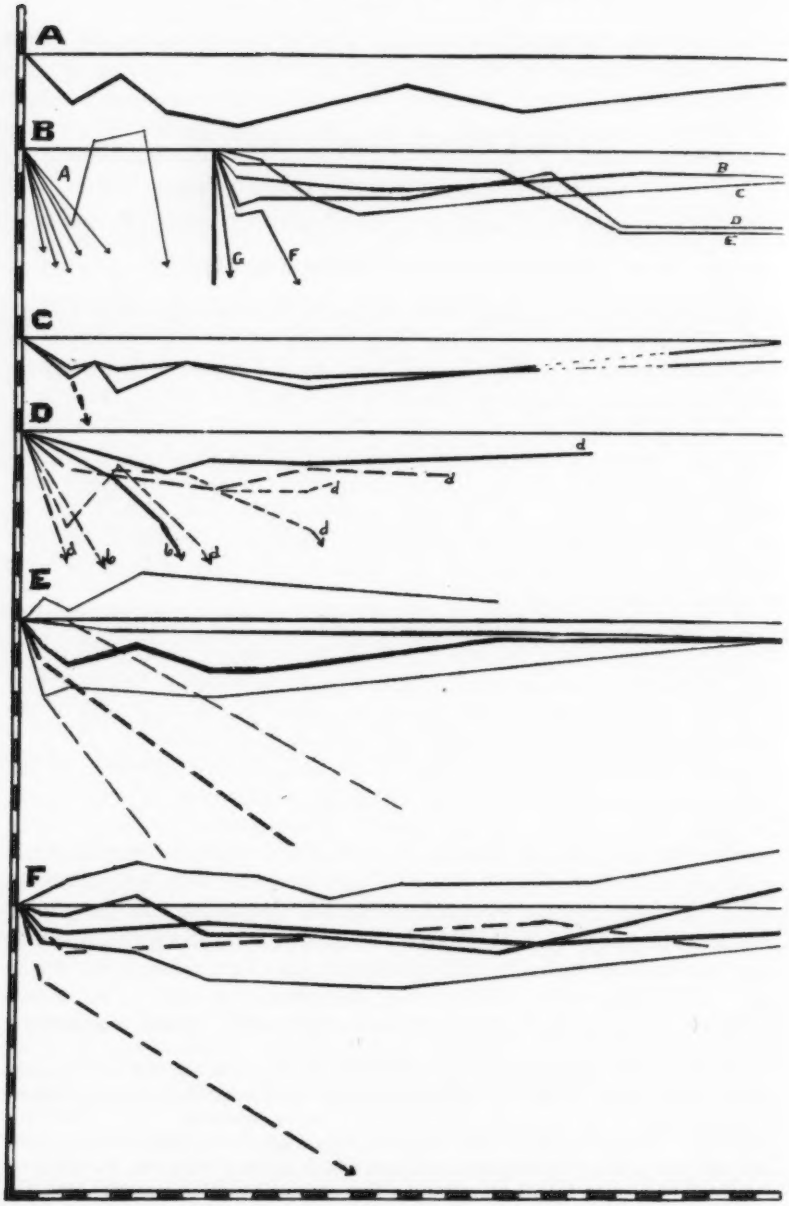

Fig. 1

soaps and fatty esters; these tests lent no encouragement. Adding radicals to the benzene ring tended to decrease its antiglucolytic properties, as did also increasing the number of halogen atoms on the ring. Tests were made with mono-, di-, tri- and tetrabenzene. The marked effect of such

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Fig. 1. The effect of halogen derivatives of benzene on blood-sugar samples. The ordinates are marked to represent deviations from the initial values by steps of 5 mgm. per 100 cc. and the abscissa is spaced as two-day intervals. The glucose concentrations have been recalculated to an initial value of 100 mgm. per 100 cc. and these points have been superimposed. Arrow-heads indicate instances where the glucose content has decreased to 30 or less mgm. per 100 cc.

A: A median curve from determinations of glucose on 6 samples of sheep blood treated with 2 cc. chlorbenzene per 100 cc. blood.

B: Curves representative of many experiments. Each tube of sheep's blood sample contained 1 gram NaF per 100 cc. Other chemicals added in conjunction with the NaF were: *A*, thymol; *B*, chlorbenzene; *C*, brombenzene; *D*, iodobenzene; *E*, trichlorethylene; *F*, trichlorbenzene; *G* tetrachlorbenzene. *B*, *D* and *E* approached the initial line beyond the time intervals represented in this graph.

C: Showing that halogen-benzene derivatives will hold the glucose content of sheep's blood near its initial level for a long time (in this test 72 days) and that other substitutions in the benzene ring have no such influence. The two solid lines represent chlor- and brombenzene and the broken line nitrobenzene. The three bottles also contained 1 gram NaF and 0.1 gram thymol per 100 cc. sample. The blood in this test was enriched by adding 120 mgm. pure commercial glucose per 100 cc. and the graph shows that the added foreign sugar is not different from the native blood glucose as to its destruction by the glucolytic mechanism of the blood. The break in the solid lines represents an interval of 47 days.

D: Showing the effect of blood preservative on the sugar content of the first 6 human samples tested in this series. The upper solid line is the median for 5 curves from diabetic blood samples with chlorbenzene (0.5 cc. in 20 cc. blood). The other solid line is a single normal specimen (no. 6) in which the chlorbenzene did not hold the blood sugar within reasonable limits. This is one of only two such instances in the history of this investigation. The six broken lines represent the results when each sample was mixed with 500 mgm. NaF and 30 mgm. thymol per 20 cc. The diabetic samples are indicated by *d*, and the single non-diabetic by *6* is from the same sample in which chlorbenzene failed to hold the sugar.

E: These curves are based on 25 consecutive diabetic cases in which the samples were treated with 10 mgm. NaF and 1 mgm. thymol per cc. The solid lines represent 12 of these in which the glucose did not drop 25 per cent in the course of 30 days; the others are indicated by the broken lines. The heavy lines are medians and the lighter lines the extremes, except one light solid line, which represents the sample which deviated the least from the initial value.

F: Showing the effect of chlorbenzene on human blood samples. The heavy solid lines represent the median values and the light lines those samples which deviated most from the initial values. The lower median line is from 6 normal blood samples and the other from samples exceeding 120 mgm. per 100 cc., excluding one which dropped to 40 mgm. on the tenth day and not considered in this graph. Together with these curves are included two normal samples treated with NaF and thymol, represented by broken lines.

halogen hydrocarbon compounds on the glucose level of blood samples at room temperature is shown by the curves in the accompanying graph.

It was our practice to plot the data for each test as the work progressed, using milligrams of glucose per 100 cc. as ordinates and the lapse of days from the sugar determination on the fresh sample as abscissae. It is not feasible to reproduce all these curves,[2] but representative cases have been redrawn to an identical scale and so placed that the initial points come together, the ordinate dimension thus showing graphically the comparative deviations from the initial glucose level.

The first curve (fig. 1, A) shows the effect of the halogen benzene compounds singly and in combination with one another. This curve is from a graph depicting 14 tests and the results are practically the same in all instances.

About this time reports of other blood preservative work appeared in the literature.[3] Among the various substances suggested sodium fluoride seemed by far the most rational and although it had been previously ruled out by a single negative test it was again given consideration. The results are shown in curves from the initial line B of the graph. Potassium fluoride and lithium fluoride were also used with identical results. None of the fluorides singly or in combination with other chemicals except halogen hydrocarbons has kept the glucose of sheep's blood from disappearing from the sample within 6 days. Sodium fluoride has, however, been included in nearly all subsequent tests and is now used by us regularly as an anticoagulant in blood sample tubes for sugar determinations, having been found useful by Sander and others for preserving human blood samples.[4]

No treatment of any kind has been found capable of holding the glucose constant within a few milligrams per 100 cc. In samples treated with halogen hydrocarbon there is, as a rule, a fall in glucose value the first two days, with a rise the next two or three days, followed by a gradual decrease to a minimum in ten or twelve days, after which the value is almost constant or perhaps slightly increased. A substantial majority of the chlorbrom-, iodo-, benzene and bromtoluene and trichlorethylene curves is of this type but not all of the curves in our collection. There may be an increase in the blood-sugar of the first two days instead of a decrease, as illustrated by the maximal curve on E. Di-, tri- and tetrachlorbenzene tests do not conform to this general type (see B).

Preference has been given to chlorbenzene in this work but brombenzene is equally effective. It is our impression that iodobenzene, bromtoluene

[2] There are several on each blood sample from two score sheep and several steers.

[3] R. H. Major, Journ. Amer. Med. Assoc., 1923, lxxxi, 1952; W. Denis and J. L. Beven, Journ. Lab. Clin. Med., 1924, ix, 674; F. V. Sander, Journ. Biol. Chem., 1923, lviii, 1; W. Denis, and M. Aldrich, Journ. Biol. Chem., 1924, xliv, 203.

[4] Personal communications from Drs. F. V. Sander and C. L. Sevringhaus.

and trichlorethylene are less useful notwithstanding the fact that in some of our curves these hold the blood-sugar as well as the first two. The amount of chlorbenzene in successful tests ranged from 0.5 to 4 cc. per 100 cc. blood. With 0.5 cc. and less the results are uncertain and in excess of 2 cc. the samples become too difficult to measure properly.[5]

The inhibiting action of these halogen-benzene compounds on glucolysis seems to last indefinitely. By C in the graph a sample is recorded which was kept under observation for more than three months. In this sample, as well as in some others, pure glucose was added to the blood in excess of the native glucose. This did not affect the type of the curve. It is also to be noted that the sugar value of a tube from the same sample but treated with sodium fluoride and nitrobenzene decreased rapidly. This emphasizes the inadequate preservative action of fluorides, also stresses the fact that it is chloryhydrocarbon which is antiglucolytic and not the benzene ring or benzene derivatives other than halogen compounds. This graph also demonstrates that the glucolytic mechanism of the blood does not differentiate between native and foreign glucose.

With these interesting data from the work on sheep's blood we proceeded to the use of human blood.[6] Securing suitable human blood samples was naturally more difficult than those from sheep and steers, and the initial glucose content more varied. The sheep bloods ranged from 70 to 90, the human specimens from 80 to 300 mgm. per 100 cc. The sheep samples could be treated immediately after the blood was drawn, whereas the human specimens were sometimes kept on ice for two days before the preservative was added. As the amount of human samples was usually such that they could rarely be divided, the odd numbers of the series were treated with a halogen hydrocarbon and the even numbers with sodium fluoride and some other compound, usually thymol.

In our experiments with human material we were surprised to meet our first failure to prevent a significant decrease in glucose with chlorbenzene—two cases out of the 153, and also to find that the sodium fluoride-thymol mixture[7] held the glucose content near the initial level in many speci-

[5] Blood samples are sent to this laboratory in 18 x 170 mm. "Comer" tubes containing 100 mgm. sodium fluoride and 0.2 cc. chlorbenzene; i.e., approximately 6.5 mgm. NaF and 14.5 mgm. C_6H_5Cl per cc. blood. More NaF does not matter but as the C_6H_5Cl exceeds 20 mgm per cc. the emulsive sediment becomes increasingly troublesome.

[6] These samples were from 153 persons, mostly diabetics from the Newark City Hospital, and for these we gratefully acknowledge our indebtedness to Dr. H. S. Martland.

[7] When sodium fluoride was discovered to hold many samples from losing glucose potassium and lithium fluoride were also tried. Between potassium and sodium there is no choice, lithium seemed less desirable. (F. V. Sander, Journ. Biol. Chem, 1923, lviii, 1.)

mens. Other laboratories have reported that the glucolytic activity of the diabetic's blood is of a lesser order than normal[8] and as most of these human specimens were diabetic this was at first considered the explanation, but this assumption proved untenable, since two normal bloods were found in one of which sodium fluoride and thymol held the blood-sugar very well and the other very badly (see broken line in F of the figure). In general the results with the fluoride, both with and without thymol, were erratic, as may be seen from our records of 25 consecutive cases treated with sodium fluoride and thymol and plotted on the initial line E of the graph. When the fluoride-thymol samples hold the results are very good indeed but as seen in many cases in which the fluoride-thymol did not prevent a decrease in sugar, the slump in glucose is remarkable. The results with halogen-benzene compounds are very much more consistent. The mechanism of the two types of preservation must be quite different. The graphs representing the data with chlorbenzene on normal blood have much of the same coefficient of variation as that for sheep's blood but the curves are not so frequently of the characteristic type described above. There is no practical difference between the normal and the diabetic curves as to form; the diabetic, however, tends to deviate less from the initial value.

SUMMARY

In an extended search for a preservative for blood-sugar it was discovered that certain halogen hydrocarbons markedly inhibit glucolysis in vitro. Among these the two most effective were chlor- and brom- benzene; next in order were iodobenzene and trichlorethylene, followed by bromtoluene and dichlorbenzene. The antiglucolytic property decreases with the increase of halogen on the benzene ring. Other substitutions in the ring also decrease this property. Methane halogen derivatives are not at all antiglucolytic.

No other substance was found which, when added to blood samples, preserved the blood-sugar as well as these halogen compounds and even these do not keep the blood-sugar within five milligrams of its initial value. The changes in glucose values of treated samples are not constant nor in one direction. The changes are most marked the first few days; after the tenth day they are almost negligible. If these changes in glucose concentration are plotted against time expressed in days they give characteristic curves which are not the same for different kinds of blood used.

Sodium fluoride and thymol will not check the decrease in glucose in sheep blood but will preserve the blood-sugar in most human blood samples, keeping them almost as well as if preserved by chlorbenzene. When this mixture is not effective the decrease in glucose is very prompt. The effec-

[8] W. Denis and U. Giles, Journ. Biol. Chem., 1923, lvi, 740.

tiveness of the fluoride-thymol mixture as a preservative bears no relation to diabetes.

The mechanism of the preservative action of halogen hydrocarbons and that of sodium fluoride plus thymol are probably very different. The halogen hydrocarbons form an emulsive sediment from which a material can be isolated which is rich in phosphorus and poor in nitrogen. The inhibition of glucolysis in vitro by chlorbenzene is such that blood samples treated with this compound may be used for clinical blood-sugar tests even after a lapse of several weeks.

The authors are indebted to Dr. William G. Exton, director of the laboratory, for encouragement and many valuable suggestions in connection with this problem; and to Dr. P. V. Wells for assistance in the statistical treatment.

A NEW METHOD FOR THE SIMULTANEOUS DETERMINATION OF MINUTE AMOUNTS OF CARBON DIOXIDE AND OXYGEN

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In connection with studies of the gas exchange of nerve a new method was recently described (Fenn, 1926) for the measurements of small amounts of carbon dioxide. The method depended upon the change in the conductivity of barium hydroxide due to the absorption of carbon dioxide. Since the publication of this method it was found that the problem in hand could be much better attacked by the measurement of oxygen volumetrically. Further description of the conductivity method has hence been considerably delayed. Ever since first use was made of this method, however, it was obvious that one of its chief advantages was that it could easily be combined with volumetric measurements so that both carbon dioxide and oxygen could be simultaneously determined. It is the purpose of this paper to show how this can be done and to describe the results of some determinations of the respiratory quotients of some isolated tissues as examples of the usefulness of the method.

The original apparatus used for the conductivity method was rather a complicated affair in which the air in the apparatus was continuously circulated through the respiration chamber, then through the barium hydrate and finally back again to the respiration chamber. Circulation was maintained by a rising and falling mercury surface actuated by a levelling bulb hung from a windshield cleaner. The necessary valves located on either side of the mercury surface were originally made all of gas. Such valves showed a tendency to stick and stop the circulation and they were finally replaced by valves made of animal membranes tied loosely over small holes in the sides of glass tubes after the pattern of Englich bicycle valves. The necessary rubber connections were effectively sealed with mercury. These worked very well and would be useful for problems in which the objects studied were suspended in solutions. In such a case air could be bubbled first through the solution with its tissue, then through the $Ba(OH)_2$ solution and finally back again to the tissue as in Osterhout's colorimetric method.

A far simpler and equally effective contrivance is the apparatus now in use which is shown in figure 1 and is adapted for tissues suspended in air. It is essentially a differential volumeter (Fenn, 1927). The two bottles are connected by a fine capillary tube carrying an index drop of kerosene. The right hand bottle is designed to hold the tissue to be studied, the other bottle serving for temperature and pressure compensation. Absorption of oxygen from the atmosphere in the tissue bottle is indicated by a movement of the index drop toward this bottle. Instead of using NaOH for the absorption of carbon dioxide a solution of $Ba(OH)_2$ is used of concentration 0.0066 M (exact strength not important). A side tube at the

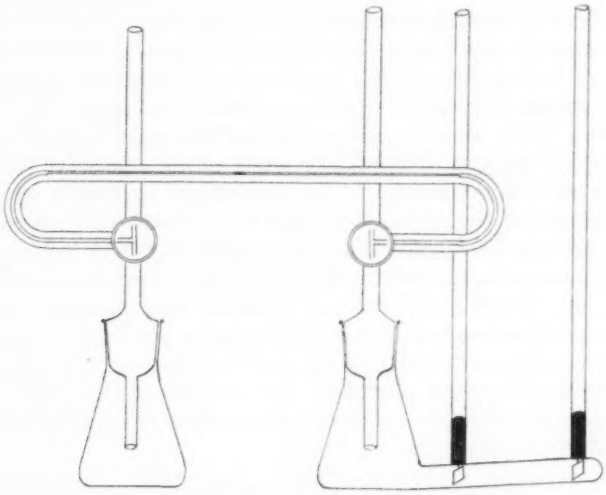

Fig. 1. Apparatus for determining oxygen volumetrically and carbon dioxide by conductivity.

bottom of the bottle is added into which the barium hydroxide runs when the apparatus is tipped slightly. Its conductivity is then measured by means of a suitable bridge using the two sealed in platinum electrodes. The side arm is 7 cm. long and holds 4 cc. The electrodes are 5 cm. apart. Except when a reading of the carbon dioxide is being taken the apparatus is so placed that the barium hydroxide is in the bottom of the bottle. Before each reading the apparatus is tilted two or three times in a uniform manner to insure complete mixing of the barium. Barium hydroxide is nearly as effective as NaOH as an absorber of carbon dioxide. Control tests have shown that after the introduction into the bottle of an amount of carbon dioxide it is absorbed at an approximate rate of 25 per cent per

minute, this rate varying of course with the size of the bottle and with the temperature. The corresponding rate of absorption for NaOH M/10 was about 40 per cent per minute. The carbon dioxide determinations therefore do not interfere in the least with the oxygen measurements. For the technique of the latter reference must be made to an earlier paper. The inside of the bottle may be painted with a solution of paraffin in ether to prevent the $Ba(OH)_2$ from sticking to the walls and so failing to mix completely.

As far as I know the sensitivities of both these methods are unequalled by any others. The oxygen is sensitive to 0.03 c.mm. if the capillary is read with an accuracy of 0.1 mm. and the carbon dioxide to 0.044 c.mm. if the conductivity reading is accurate to 0.02 per cent. We have used still smaller capillary tubes for oxygen making this method twice as sensitive and the carbon dioxide method could be made somewhat more sensitive by using smaller amounts of solution in a smaller side-arm.

Warburg (1926) has a manometer method for both CO_2 and O_2 depending upon their relative solubilities in water which is sensitive to 0.2 mm.[3] CO_2 may also be determined in the manometric apparatus by separate determinations of the original and final CO_2 content of the tissue. The attempt has also been made (Fenn) to determine CO_2 by volume change measurements with and without NaOH making a correction for the retention of CO_2. Slater (1927) recently described an apparatus in which O_2 is determined manometrically and CO_2 by the katharometer. In this case however the O_2 is sensitive to 1 c.mm. and the CO_2 to only 0.5 c.mm. which is not sufficient for many purposes. The methods of Slater and Warburg have the advantage of permitting measurements to be made on tissues kept at a finite CO_2 tension.

Calibration of the conductivity method. The change in conductivity of a solution of $Ba(OH)_2$ should be proportional to the slope of the graph resulting from plotting the specific conductivity, χ as ordinates against the concentration, C, as abscissae. Two sets of data on the conductivity of $Ba(OH)_2$ have been found in the tables of Landolt Bornstein in volume II p. 1093, and in the "Erster Erganzungsband," p. 603. The former give values considerably lower than the latter. The latter have been adopted as the more reliable because they are more consistent both with themselves and with my own experimental values. Unfortunately the figures given are not very numerous for the range of concentrations used in my methods. In order to obtain more points from which to derive the shape of the curve I have had recourse to a method of graphical interpolation on a curve in which the equivalent conductivity is plotted against the logarithm of the concentration, C. Corresponding values of conductivity and log C were read off on this curve. From these data in turn values of C and of the specific conductivity χ_{25} (equivalent conductivity $\times$ equivalent concen-

tration) were calculated. By subtracting successive values from each other one obtains the slope of the curve in terms of $\frac{\Delta\chi}{\Delta C}$ for each successive interval. Now 1 c.mm. of dry CO_2 at 0°C. and 760 mm. pressure is 4.46 $\times 10^{-8}$ mols. The change in conductivity to be expected when 1 c.mm. dry CO_2 is absorbed in a cell containing 3.97 cc. of $Ba(OH)_2$ is then $\frac{4.46 \times 10^{-8}}{0.00397} \times \frac{\Delta\chi}{\Delta C}$. The values of $\Delta\chi_{25}$ per c.mm. CO_2 so calculated are plotted in the middle graph (dots) in figure 2, against the specific conductance of varying concentrations of $Ba(OH)_2$ as abscissae. Corresponding

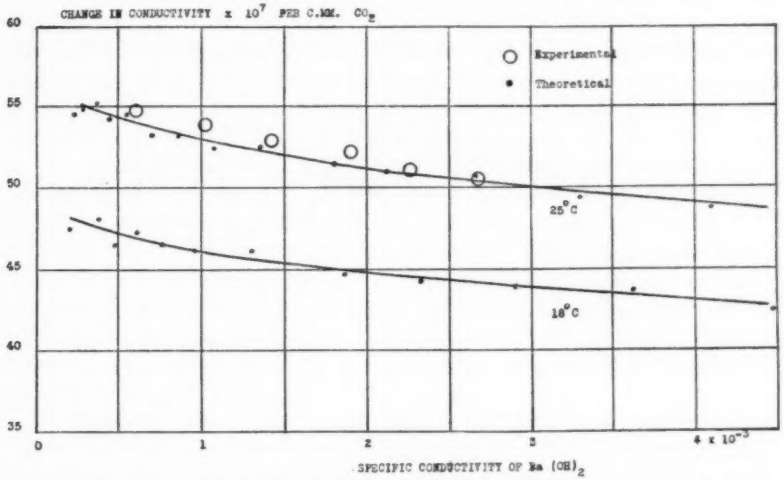

Fig. 2. Calibration of the conductivity method. Ordinates represent the change in specific conductivity of $Ba(OH)_2$ at 25° when 1 c. mm. dry CO_2 at N.T.P. is absorbed. Abscissae represent the specific conductivity of the $Ba(OH)_2$ solution.

figures for 18°C. are plotted in the lower graph. From these graphs it is easy to calculate how much change in CO_2 corresponds to an observed change in conductivity of a solution of a given strength as determined by its conductivity.

To avoid any possibility of error in these calculations a direct calibration of this method was thought necessary. We have therefore introduced known amounts of carbon dioxide into our cell and have observed directly the resulting change in conductivity. The results of these observations are recorded as circles in the upper graph of figure 2 and it is seen that they follow the theoretical curve fairly closely, although they are consistently slightly higher. The reason for this discrepancy we have not

been able to discover.[1] The experimental figures are supported by a large number of preliminary points obtained before the technique was thoroughly mastered. Three corrections have been applied to these figures before plotting: *1.* They have been multiplied by 9.28, this being the constant of our cell. *2.* They have been multiplied by 0.992 to reduce them from 25.4°C. at which they were observed to 25°C. *3.* An amount $\chi_{25} = 0.7 \times 10^{-4}$ has been subtracted from each abscissa, this being the specific conductivity due to dissolved $BaCO_3$, distilled water and any impurities which the solution might contain in small amounts. This last correction is determined by precipitating all the Ba as $BaCO_3$ and measuring the conductivity. In doing this it should be remembered that $BaCO_3$ is soluble in excess of CO_2 so that if the solution is saturated with CO_2 the precipitate will redissolve and the conductivity will again increase (Haehnel, 1924).

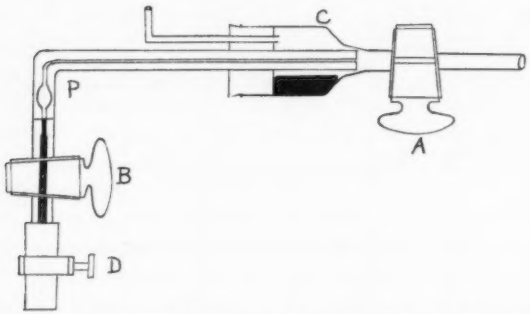

Fig. 3. Sampling pipette and apparatus for sampling CO_2 for calibration.

The introduction of the carbon dioxide into the cell for the calibration presents some difficulty and the method adopted was suggested by one used by Parker (personal communication) for calibrating his colorimetric CO_2 apparatus. The apparatus figure 3 consists of a sampling pipette, *P*, fitted with a stopcock, *B*, and a rubber bulb, *D*. This pipette is filled with mercury and inserted into the chamber *C* through which pure carbon dioxide is kept passing, entering through *A*. By releasing the clamp on the rubber bulb of the sampling tube, carbon dioxide is drawn into it until the mercury reaches a mark above the bulb, when the stopcock is closed. The whole apparatus is now submerged in a water bath, the carbon dioxide is turned off and time is allowed for temperature equilibrium to be

[1] It may be calculated from the formula of Fricke (Phys. Rev., 1924, xxiv, 575) that the precipitate of $BaCO_3$ equivalent to 1 c. mm. CO_2 would change the conductivity less than 0.2 per cent.

attained. After 5 to 10 minutes the apparatus is tipped to the vertical position so that the mercury in the chamber C runs down and seals off the end of the sampling pipette. The rubber bulb on the pipette is then released a little and the stopcock opened until a short mercury plug is drawn into the tip of the pipette. The whole pipette is then removed from C and inserted through a rubber stopper into the bottle of the respirometer already containing 3.97 cc. of 0.0066 M barium hydroxide. The respirometer bottle with its pipette is left submerged to the neck in a water bath and the conductivity of the barium hydroxide is measured at intervals until a constant minimum rate of change is obtained. A lower rate is always reached with a glass stopper ($\chi_{25} = 2 \times 10^{-8}$ per min.) than with a rubber one (1×10^{-7} per min.). When this minimum rate is attained the sample of carbon dioxide is expelled into the bottle and conductivity readings are again taken at intervals until the basal rate is again reached. From the change in conductivity (subtracting the basal rate) one can calculate the change to be expected when 1 c.mm. of dry carbon dioxide at 0°C. and 760 mm. pressure is absorbed. By more or less continuous tipping of the apparatus absorption is made 90 per cent complete in 3 minutes so that the correction for the basal rate (about $\chi_{25} = 1 \times 10^{-7}$ per min.) becomes relatively small. The purity of the CO_2 was tested by immersing the pipette containing a typical sample in NaOH and carefully expelling the mercury plug. All the CO_2 was then absorbed as the NaOH was drawn into the capillary. The CO_2 was passed through distilled water at room temperature to give it a vapor pressure of 22 mm. before using.

Simultaneous absorption of any possible ammonia given off by pieces of tissue under observation would not appreciably affect the CO_2 readings. It has been found experimentally and from theoretical considerations that the dissociation of NH_4OH in the presence of $Ba(OH)_2$ is so small that the absorption of 1 c.mm. of NH_3 would make an error of less than 1 per cent in the determination of 1 c.mm. of CO_2.

Oxygen measurements. In order to determine accurately the absolute values of dry oxygen at N.T.P. certain precautions must be observed. First, the volumes of the air spaces in the two bottles of the respirometer must be equal or be known. Secondly, the vapor pressure must be the same in both bottles or the differences must be known. To take into consideration the vapor pressure and the case where the volumes of the bottles are not equal the theory must be reconsidered.

Let V_r and V_c be the volumes of the respiration and the control bottles respectively. Let p be the initial pressure in both bottles and p_1 be the pressure after x cc. of dry gas at the temperature and pressure obtaining in the apparatus are used up in V_r. Pressure in the two bottles is equalized

TABLE 1

Oxygen consumption and carbon dioxide output in cubic millimeter per gram per minute at varying intervals after dissection

TIME	O_2	CO_2	R.Q.
Expt. 1. Ileofibularis muscle 38 mgm.			
hours			
1	3.25	2.61	0.80
Expt. 2. 2 Ileofibularis muscles 250 mgm.			
½	2.43	2.15	0.89
1½	1.89	1.92	1.02
3½	1.70	1.71	1.01
Expt. 3. 2 Semitendinosus muscles 132 mgm.			
½	3.48	3.45	0.99
2	2.71	2.55	0.94
4½	1.95	1.87	0.96
Expt. 4. 2 Semitendinosus muscles 168 mgm.			
½	3.77	3.24	0.86
1½	3.09	2.53	0.82
Expt. 5. 4 Sciatic nerves 132 mgm.			
1	1.69	1.90	1.13
2	1.58	1.75	0.90
3½	1.49	1.84	0.81
4½	1.49	1.71	0.87
5½	1.43	1.77	0.81
6½	1.37	1.66	0.83
7½	1.32	1.68	0.79
Expt. 6. 6 Sciatic nerves 127 mgm.			
1	1.33	1.76	1.32
2	1.60	1.53	0.96
2½	1.50	1.41	0.93
3½	1.56	1.36	0.87
Expt. 7. Beating heart 125 mgm.			
1	2.72	3.42	1.26
2½	2.90	3.31	1.14
4½	2.86	2.83	0.99
6	2.82	2.76	0.98

TIME	O_2	CO_2	R.Q.
Expt. 8. Non-beating heart 192 mgm.			
hours			
1	1.73	1.88	1.09
2	1.58	1.60	1.01
Expt. 9. Beating heart 450 mgm.			
1	1.47	3.56	2.42
2	1.34	1.91	1.42
5	2.27	2.60	1.15
5½	2.47	2.88	1.17
6½	2.62	2.60	0.99
Expt. 10. Skin of leg 254 mgm.			
1	2.08	3.29	1.58
2	2.52	2.23	0.88
2½	2.56	2.34	0.91
Expt. 11. Skin 147 mgm.			
1	3.03	3.09	1.02
2	2.84	2.39	0.84
Expt. 12. Small intestines 197 mgm.			
½	4.02	5.28	1.31
1	4.71	4.05	0.86
1½	4.82	4.42	0.92
2½	4.87	4.37	0.90
3	5.00	4.16	0.83
4	4.91	4.26	0.87
4½	4.86	3.98	0.82
Expt. 13. Stomach 210 mgm.			
½	3.73	5.05	1.35
2½	2.77	3.03	1.09
3½	2.84	2.72	0.96
4½	2.77	2.57	0.93
5½	2.72	2.35	0.86
6½	2.61	1.71	0.65

by a movement of the index drop over a volume of the capillary equal to d. The vapor pressure in both bottles is y. Then

$$\left(V_r - x - \frac{yd}{p}\right) p = (V_r - d) p_1$$

and

$$\left(V_c + \frac{yd}{p}\right) p = (V_c + d) p_1$$

whence

$$\left(V_r - x - \frac{yd}{p}\right)(V_c + d) = \left(V_c + \frac{yd}{p}\right)(V_r - d)$$

and

$$x = d\left(\frac{V_c + V_r}{V_c + d}\right)\frac{(p - y)}{p} = \frac{d(1 + f)}{f}\,\frac{p - y}{p}$$

where $f = \dfrac{V_c}{V_r}$ and d is negligible compared to V_c.

For the purpose of obtaining absolute values a further correction has been introduced to allow for the change in the volume of the capillary when its walls are wetted with kerosene. My capillary held 0.001637 cc. per centimeter. A kerosene drop introduced into one end of this tube when clean and dry loses 1.5 mm. in length after travelling 11.5 cm. The corrected figure is therefore $\dfrac{11.5 - 0.15}{11.5} \times 0.001637$ or 0.001616 cc. per cm.

RESULTS. In thirteen experiments the respiratory quotients of isolated tissues of frogs in an atmosphere of pure O_2 have been recorded and the results are listed in table 1. The figures show that determinations of the R.Q. for single periods starting shortly after temperature equilibrium is reached in the water bath are seldom representative of the values finally reached. There is a very general tendency for the R.Q. to diminish during the first hour or two. This diminution is due both to a decrease in the CO_2 which is practically invariable and sometimes to an increase in the oxygen in addition. It should be emphasized that these figures represent the CO_2 *liberated* at zero CO_2 tension. Any accumulation of an acid such as lactic acid or of a base such as ammonia would cause a liberation of preformed CO_2 or a retention of formed CO_2. The only way in which this error is properly reckoned with is by the determination in two samples of the CO_2 content of the tissue before and after an experiment. The low values for the R.Q. here recorded certainly suggest, as others have also found, that the foodstuff burned is not purely carbohydrate.

The initial high CO_2 rate may be partly accounted for by a slight increase in the resistance of the $Ba(OH)_2$ which takes place in the absence of tissue.

This change is, however, small, being equivalent to 0.01 c.mm. during the first hour and falling to 0.001 c.mm. per minute after two hours. It may also be due to glycolysis in the interior of the tissue if the supply of oxygen there is inadequate.

SUMMARY

By a combination of the volumetric method for oxygen and the conductivity of barium hydrate method for carbon dioxide an apparatus is constructed which determines both carbon dioxide and oxygen simultaneously and continuously in ten minute periods in 0.1 gram samples of tissue with a sensitivity of 0.03 to 0.05 c.mm. Some typical results are described.

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Since the above was written I have made the CO_2 method about 4 times as sensitive by using only 1 cc. of $Ba(OH)_2$ in a much smaller bottle. The bottle has no side arm but the electrodes are simply sealed in at the bottom of the bottle on opposite sides and the conductivity of the solution is read without tipping. This works quite as well as the tipping method. By putting a cup in the bottom of the bottle and gently shaking the apparatus continuously the CO_2 output of materials in solution can be followed. I have used this method successfully for following the increased gas exchange of both nerves and muscles during stimulation. The rates of CO_2 output so measured are if anything more regular than the oxygen rates.

Bayliss has recently published a paper entitled "A conductivity method for determining CO_2" without realizing that it had been used before. Bayliss, Biochem. Journ., xxi, 662, 1927.

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XXII. Adrenin Secretion on Exposure to Cold, Together With a Possible Explanation of Hibernation

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Hibernation and the effects of cold on the organism have been made the subject of age-long scientific inquiry. Since Claude Bernard's observations in 1855 on the reduction of the liver glycogen in cooled rabbits and guinea pigs, many investigators have given attention particularly to the influence on carbohydrate metabolism of low external temperatures. A brief review to 1921 is given on this phase of the investigation by Morita. Fresh stimulus has now been infused into the subject with the advent of insulin.

A recent series of papers by Cassidy, Dworkin and Finney (1925–1927) is of noteworthy interest. In dogs and cats insulin was found to reduce the blood sugar with the same rapidity whether the body temperature was 38° or as low as 25°; there was no tendency of the sugar concentration to return to normal, however, in animals at the subnormal temperature. It was also found possible to produce a condition simulating hibernation in cats, dogs and woodchucks by the combined action of cold and insulin. Very low respiratory quotients were observed in these cases. The injection of glucose brought about shivering movements, and there followed a rapid termination of the dormant condition. Adrenalin and pituitrin, as well as various sugars, were observed to restore shivering in animals in which the reflex had previously been abolished during insulin hypoglycemia. These very suggestive findings have not yet been given confirmatory support.

The ability of a hibernating animal (*Arctomys monax*) to recover spontaneously after artificial cooling from as low a body temperature as 3° was demonstrated by Tait and Britton (1923). Homoiothermic animals (cats and guinea pigs) may be restored to normal after reducing the deep rectal temperature to 16°, and spontaneous recovery from a body temperature of 19° may occur in the cat in ten to twelve hours (Britton, 1922). The mechanisms involved in the performance of such remarkable thermogenic feats were not at the time inquired into by these authors. A great deal of evidence indicates, however, that an augmented activity of the

endocrine organs is associated with environmental and body temperature changes. Analysis of this evidence has been made in a recent paper by Cannon, Querido, Britton and Bright (1927). These workers also present experimental proof that an accelerated adrenin secretion from the adrenal glands serves to protect the organism against dangerous heat loss on exposure to cold surroundings, and show that the same conditions which increase adrenal secretion in lower animals likewise increase metabolism in man. These findings have an important bearing, it will be observed, on the question of true chemical augmentation of metabolic activity.

The hyperglycemia which occurs in rabbits when their temperature is lowered by artificial cooling has lately been shown by Geiger (1927) to be prevented by bilateral splanchnectomy. This operation, it should be noted, markedly affects the systemic blood pressure and, since it cuts off the hepatic nerve supply and largely eliminates the nerve impulses to the adrenal medulla, it also suppresses the discharge of sugar from the liver. Lawrence (1927) has suggested that the blood-sugar rises observed on cooling animals may indicate "adrenaline reactions."

During a recent investigation on the effects of nervous influences on the secretion of insulin (Britton, 1925) it was observed that slight cooling and subsequent shivering frequently occurred in cats under amytal (iso-amyl-ethyl-barbituric acid) narcosis. These conditions were found to be accompanied by changes in sugar content of the blood. A few of the experiments which were carried out indicated that secretion from the adrenal glands might be a potent factor in producing these changes, and further work was undertaken to give this suggestion a crucial test. To evoke if possible well-marked reactions and thus derive significant data, it was decided to establish a predicament calling for immediate and urgent activity on the part of the organism if it were to survive. This was provided by the exposure of animals that had been insulinized, and were showing hypoglycemic symptoms, to dangerously cold surroundings. Changes in blood sugar and indications of adrenin secretion in these animals were thereupon studied.

Procedure. Vigorous, full-grown cats were used for the experiments. In all cases they were fed on the day previous to being used and were kept fasting 12 hours over night. Initial blood samples for making the sugar analyses were taken from the marginal ear vein; later samples were drawn from the femoral artery by an inserted glass cannula. The Folin-Wu method (1920) was used for determining the blood-sugar concentration. Insulin (Lilly) was given by subcutaneous injection immediately after the first blood specimen was taken. The amounts used are noted in table 1.

After the blood sugar had reached the convulsive level (from two to four hours after insulin) the animal was immersed to the neck in a water bath and thereby cooled. Water at room temperature was employed at the

outset, and as the animal became more accustomed to its environment the bath temperature was reduced by the addition of ice. Thus the temperature of the water varied between 20° and 5°. This method generally allowed the immersion and cooling of hypoglycemic animals with little apparent discomfort. In a few instances when slight distress was shown a small amount of ether or of amytal—30 mgm. per kilo, intraperitoneally, of the latter—was administered (see tables 1 and 2). Ten minutes or so after starting the cooling process the animal's body temperature had fallen a few degrees below normal, and a condition resembling deep slumber supervened.

The period of exposure to cold usually lasted from one-half to one hour. Following this the animal was well dried with towels and arranged for

TABLE 1

The effects of exposure to cold on insulinized (hypoglycemic) cats. The adrenal glands were intact in all cases

EXPERIMENT NUMBER	DATE	INSULIN GIVEN (UNITS PER KILO)	BLOOD SUGAR PER 100 CC.			TIME EXPOSED	RECTAL TEMPERATURE AFTER EXPOSURE	SHIVERING DURING INITIAL STAGES OF EXPOSURE	ANESTHETIC USED
			Before insulin given	After reduction by insulin	After exposure to cold				
			mgm.	*mgm.*	*mgm.*	*minutes*			
1	9/16/25	3	122	53	75	30	34.1	Moderate	None
2	9/18/25	3	128	53	89	130	25.8	Vigorous	Ether
6	9/29/25	4	102	47	202	80	17.5	Vigorous	None
9	10/ 5/25	4	171	67	205	25	24.2	Moderate	Amytal
14	10/12/25	4	121	71	143	35	18.5	Moderate	None
16	10/14/25	4	114	28	90	40	22.6	Moderate	None
23	1/15/26	2	150	79	197	55	27.3	Vigorous	None
24	1/15/26	2	173	55	267	50	23.5	Vigorous	None

observation. Further blood specimens were taken, and kymographic records of blood pressure and pulse, respiration, and shivering movements were made at intervals. The peripheral tissues for several centimeters below the surface were found to be much colder than the internal parts; therefore, deep rectal temperatures (the thermometer bulb being introduced into the rectum for six or eight centimeters) were in all cases taken.

RESULTS. In the majority of cases (see table 1) it was observed that the body temperature of the hypoglycemic animal was reduced 15° to 20° below normal within an hour after exposure to cold. The reduction occurred whether a small amount of anesthetic was used, as in a few instances, or not.

Table 1 presents the results of experiments carried out on *animals with the adrenal glands intact.* Of particular significance are the large increments

in blood sugar and the occurrence of shivering in all cases during exposure. An emergence from a hypoglycemic to a markedly hyperglycemic condition was observed in several of the experiments, e.g., in experiment 6 the change was from 47 mgm. to 202 mgm. per cent, in experiment 9 from 67 mgm. to 205 mgm. (this increase occurring during only 25 minutes' exposure to cold), and in experiment 24 from 55 mgm. to 267 mgm. The range of increase of blood sugar in eight experiments was from 22 mgm. to 212 mgm. per cent. No precise relation was apparent between the temperature to which the animal was cooled and the change in the blood-sugar level.

Commonly it was observed that slight shivering movements commenced a few minutes after immersion and gradually, as the rectal temperature reached 33° to 30°, the tremors became more pronounced. Vigorous shivering was usually manifested during the period of cooling from 30° to 25°; in a few cases it disappeared before 25° was reached. During this period, furthermore, there developed marked hypertonicity of the skeletal musculature, and both the fore and the hind limbs were often (with the exception of the tremors) held quite rigid. As the body temperature approached 20° shivering subsided and coincidently respiratory distress usually became apparent. These later phenomena served, indeed, as criteria for immediate withdrawal of the animal from its cold environment, and the rapid disintegration of bodily activities which sets in at lower temperatures was thereby prevented.

In several instances the convulsive seizures which occurred during severe insulin hypoglycemia were present on initially exposing animals with the adrenals intact to cold. Invariably, however, complete inhibition of the convulsions took place on *lowering* the body temperature. A typical case is given below.

Experiment 2. Cat, female, 1.7 kilo.

September 18, 1925. 10:20 a.m., blood sugar 128 mgm. per cent; 10:25, insulin, 3 units per kilo given subcutaneously. 12:30 p.m., convulsions. 1:00, convulsions. 1:45, blood sugar 53 mgm. 2:05, convulsions. 2:20, animal placed in cold bath; no distress shown. 2:35, marked shivering, struggling; ether given occasionally. 3:45, rectal temperature 27.5°; continued vigorous shivering, muscles hypertonic. 4:30, blood sugar 89 mgm., rectal temperature 25.8°, pulse 98, respiration 17; marked shivering, no convulsions; taken out of bath. 5:55, temperature 28.8°, pulse 122, respiration 22; vigorous shivering and general body movements. Later the animal appeared to be recovering consciousness; it was killed with ether.

No convulsions were observed in the above experiment during prolonged exposure (for more than two hours) of the animal to cold. Indeed, after withdrawing it from the bath there appeared general signs of returning consciousness—temperature, pulse rate and respiration rapidly approached the normal levels, and the coincident bodily movements became vigorous.

These reactions, as well as the ability to recover the normal blood sugar level, indicated that very potent reserve forces are possessed by and also available to animals with the adrenal glands intact.

The reactions to cold of hypoglycemic *animals without active medulli-adrenal tissues* are reported in table 2. To test the possibility of eliciting responses similar to those obtained from normal animals, the operations were carried out by various methods. In experiments 11 and 12 the adrenal glands were tied off (*via* anterior abdominal incision) by means of mass ligatures during the occurrence of hypoglycemia produced by insulin. This procedure enabled the operation to be carried out very rapidly (usually in six to eight minutes) with the employment of only a small amount of anesthetic. The blood sugar was determined before and after

TABLE 2

The effects on insulinized (hypoglycemic) cats of exposure to cold. The adrenal glands were inactivated before exposure in all cases

EXPERIMENT NUMBER	DATE	INSULIN GIVEN (UNITS PER KILO)	BLOOD SUGAR PER 100 CC.					TIME EXPOSED	RECTAL TEMPERATURE AFTER EXPOSURE	ANESTHETIC USED
			Before insulin given	After reduction by insulin	After both adrenals inactivated	Before exposure to cold	After exposure to cold			
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*minutes*		
11	10/ 8/25	4	173	64	48	33	35	35	23.8	Amytal
12	10/ 9/25	4	110	49	54	35	42	35	23.1	Amytal
19	11/ 9/25	4	137	54		54	56	40	25.3	Ether
20	12/19/25	3	109	32		32	34	60	24.4	None
21	12/19/25	3	99	48		48	50	55	24.8	None
22	1/14/26	2	102	41		41	53	60	25.0	None

Shivering did not occur in any case during the exposure to cold.

operation and an interval was allowed before exposure to cold. In experiment 19 the adrenals were ligated shortly before the injection of insulin. The animals used in experiments 20, 21 and 22, however, were subjected to medulliadrenal inactivation (by excision of the right gland and denervation of the left) over a week in two cases, and five weeks in the last case, before the reactions to low temperatures were assayed.

In comparison with the normal control cases reported in table 1, noteworthy differences in response to cold appeared in the animals in which the secretion of adrenin had been suppressed. The changes in blood-sugar level (see table 2, figure 1) were in all instances negligibly small—the greatest increase observed was that of 12 mgm. per cent (in experiment 22) at the end of an exposure period of one hour. The average elevation of the blood sugar was slightly less than 4 mgm. per 100 cc. In no instance,

moreover, was the occurrence of shivering observed during immersion in the cold bath. Feeble clonic movements of the fore and hind limbs were sometimes evident, and muscle tonus also was usually increased. Convulsions disappeared at body temperatures of 20° to 25°. The following protocols are illustrative of the foregoing.

Experiment 20. Cat, female, 2.3 kilos.

December 11, 1925, adrenals inactivated (right gland excised and left denervated); recovery uneventful.

December 19, 11:10 a.m., fasting blood sugar 109 mgm. per cent. Insulin, 3 units per kilo, given at 11:50. 2:20 p.m., blood sugar 32 mgm.; animal had two convulsions

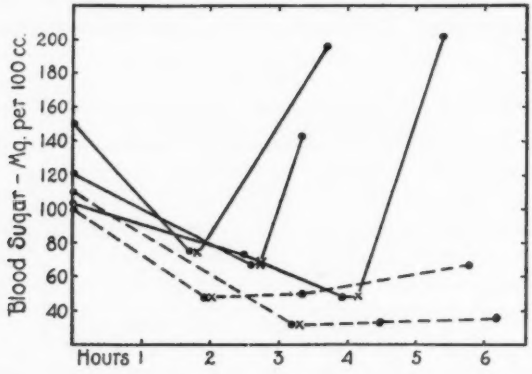

Fig. 1. Curves showing typical increases in blood-sugar level in normal cats (continuous lines) which were subjected to cold exposure following insulin hypoglycemia. In animals with the adrenal medulla inactivated (dash lines) under similar conditions, negligible changes in blood sugar were observed. Insulin given at 0 hours; animals exposed to cold at *X*. See tables 1 and 2.

just after blood was taken. Exposed to cold water bath at 2:25; no anesthetic used; slight discomfort shown; no shivering. 2:45, limb and trunk muscles tonic; no shivering. Animal taken from bath at 3:25, dried and placed on observation table; rectal temperature 24.4°, pulse 70; no convulsions. 3:35, blood sugar 34 mgm. 4:30, blood sugar 35 mgm.; muscle hypertonus; no shivering; temperature 23.7°. 5:20, blood sugar 36 mgm.; temperature 23.4°. Spontaneous recovery did not appear probable. The animal was destroyed.

Experiment 21. Cat, male, 2.9 kilos.

December 11, 1925, adrenal glands inactivated as in experiment 20. Animal made good recovery.

December 19, 11:25 a.m., fasting blood sugar 99 mgm. per cent. Insulin, 3 units per kilo, given at 11:51. 1:20 p.m., blood sugar 48 mgm.; respiration shallow and rapid. Exposed to cold bath at 1:25; no anesthetic given; slight struggling. 1:40, no shivering; muscles hypertonic. 2:22, withdrawn from bath; rectal temperature

24.8°; no shivering. 2:44, blood sugar 50 mgm.; marked hypertonus. Cat put on warming pad and normal body temperature restored.

December 20, animal took food; appeared well.

Experiment 22. Same cat as used in experiment 21; weight 3.4 kilos; condition excellent.

January 15, 1926, 9:45 a.m., fasting blood sugar 102 mgm. per cent. Insulin, 2 units per kilo, given at 9:55. Convulsions occurred at 11:22, at 11:26 and at 11:30; panting and salivation. 11:32, blood sugar 41 mgm. Animal placed in cold water bath at 11:35; no anesthetic; slight struggling. 12:00 m., occasional clonic limb movements; no shivering. 12:35 p.m., animal taken from bath; rectal temperature 25.0°; no convulsions. 2:00, temperature 24.2°; no shivering. 3:00, no shivering. 4:25, blood sugar 53 mgm. Glucose solution given at 4:35. Animal restored to normal.

In the absence of secretion from the adrenal medulla, therefore, the organism, though subjected to extreme danger, is unable to mobilize effectually its glycogen reserves, and also does not emerge without artificial aids from the profound depression resulting from exposure to cold.

In two further medulliadrenal inactive cases in which the blood-sugar levels were within normal limits before exposing the animals to low temperatures, slight shivering movements were observed within a short period after immersion. No significant changes, however, occurred in the blood-sugar concentration in these cases.

Effects of adrenalin on cooled animals. The observations that in the presence of adrenin secretion animals exposed to cold undergo vigorous shivering and show marked changes in blood sugar led to an inquiry into the effects of adrenalin on cooled animals. In table 3 are listed cases in which the glycemia was at the normal or a higher level before the injection of adrenalin hydrochloride; the body temperature was in all instances very much reduced and the shivering reflex was suppressed. Intravenous administration of small amounts of dilute adrenalin invariably reproduced shivering movements, however, as well as increases in the blood-sugar percentage. Usually the tremors began within a few minutes after injection, increased in intensity in the course of ten or fifteen minutes, and then gradually subsided, although the blood sugar stood at a higher level than before the administration of adrenalin. The phenomena occurred after ligation of the adrenals as in unoperated animals.

No significant changes in body temperature following adrenalin were noted in the preceding group of cases. In one instance in another series of experiments (table 4, Sept. 18) a sharp rise of over a degree in rectal temperature was noted a few minutes subsequent to adrenalin injection. Coincident with the rise were vigorous shivering movements and a marked augmentation of the blood-sugar percentage from the convulsive level. The remaining experiments listed in table 4, in all of which hypoglycemia

was initially present, further demonstrate the effects of adrenalin in producing glycogenolysis and restoring the shivering reflex.

The injection of glucose solution was similarly observed to reëstablish promptly the shivering reflex in hypoglycemic animals that had undergone a reduction in body temperature to 20° to 25°. Ringer's solution was ineffective.

TABLE 3

Effects of adrenalin on animals after exposure to cold and suppression of the shivering reflex at low body temperatures. One case, adrenal glands inactive; others, adrenals active

DATE	TIME	BLOOD SUGAR	RECTAL TEMPERATURE	ADRENALIN (1:50,000) GIVEN	REMARKS
		mgm. per cent		*cc.*	
10/ 1/25	5:15 p.m.	322	23.4		(*Adrenals intact*) Animal quiet
	5:30		23.4	1.28	Slight shivering at 5:38
	6:00			1.92	Shivering after adrenalin injected
	6:09		23.4	1.92	Slight shivering
	6:25	400	23.6		Shivering still present
10/ 1/25	8:20 p.m.	406	21.3		(*Adrenals intact*) Quiet
	8:24			1.92	Shivering few minutes after adrenalin given
	9:10	413	21.0		
10/ 5/25	3:15 p.m.	205			(*Adrenals intact*) Quiet
	3:45		22.6	0.80	Shivering just after adrenalin given
	3:50				Marked shivering
	4:00	234	22.4		Moderate shivering
10/15/25	7:15 p.m.	80	25.2		(*Adrenals ligated*) Animal quiet
	7:19			0.68	Slight shivering at 7:25
	7:30	164	25.2		Marked shivering; struggling

The adrenal mechanism and hibernation. Evidence which has been adduced by Mann (1916) indicates that the adrenal glands in hibernating animals (spermophiles) undergo fairly definite and uniform seasonal fluctuations. As winter approaches the size of the adrenals decreases, and histologically all the cells appear to become smaller. During hibernation the medullary cells are observed to be shrunken, but the lipoid content of the cortex does not change greatly. In the spring, significantly, the ad-

renal shows a gross increase in size, and on histological examination all the cells of both the cortex and medulla are found to be swollen. Inconstant and slight changes were usually found in other internally secreting organs. In two cases in which the right adrenal had been previously excised the animals became dormant at rare intervals during the hibernating season. A large number of experiments involving other glandular structures were performed without definite result. It was impossible to state from the

TABLE 4

Reactions to adrenalin of hypoglycemic (insulinized) animals in which the shivering reflex was abolished after prolonged exposure to cold

DATE	TIME	BLOOD SUGAR	DEEP RECTAL TEMPERATURE	ADRENALIN (1:50,000) INJECTED	REMARKS
		mgm. per cent		*cc.*	
9/16/25	6:15 p.m.	51	26.2		Animal quiet
	6:30			1.60	Shivering started at 6:33
	6:35	60	26.2		Vigorous shivering
9/18/25	8:40 p.m.	46	28.3		No shivering
	8:47			0.70	Shivering marked at 8:50
	9:00	104	29.5		Vigorous shivering
10/ 7/25	4:25 p.m.	31	24.2		Quiet
	5:05			0.28	No shivering
	5:14			0.28	Weak movements
	5:21			0.28	Shivering
	5:30	64	24.2		Vigorous shivering at 5:25
10/ 7/25	7:25 p.m.	34	22.0		Quiet
	7:47			0.56	Quiet
	8:02			0.56	Slight shivering
	8:10			0.28	Shivering present; activity
	8:30	67			

observations, Mann concluded, that some of the ductless glands were not of importance in relation to hibernation.

Other workers have considered the question from a different viewpoint. As pointed out by Cushing and Goetsch (1913, 1915), a train of symptoms similar to those accompanying the state of hibernation is observed in hypopituitarism and experimental hypophyseal deficiency. Microscopic changes were found by these authors in the anterior pituitary lobe in woodchucks during the dormant period, and it was conceived that although a pluriglandular inactivity might bring about hibernation, a particular rôle should possibly be ascribed to the pituitary body. In those instances

in which the adrenal glands also were examined by Cushing and Goetsch, however, it is noteworthy that very significant changes, suggestive of diminished activity during hibernation, were manifest.

Vignes (1913) considered that the so-called hibernating gland (*la masse hibernale*) may be physiologically important as a protein economizer during the period of winter sleep. A review on hibernation by Rasmussen (1916) gives further consideration to theories on this phase of the subject.

Of peculiar interest and importance are the events accompanying the process of awakening in hibernating animals. The most striking phenomenon is the rapid rise in metabolic rate (Pembrey, 1901), accompanied by an extraordinary increase in body temperature; in the ground-squirrel (*Citellus tridecemlineatus*), for instance, a rise of 30° in an hour has been reported (Johnson, 1924), while the temperature of the dormouse sometimes "rushes up many degrees in a few minutes" (Pembrey and White, 1896). The respiratory rate and heart beat simultaneously show a marked quickening. In very torpid animals these changes may, at the outset, take place only gradually. Increased muscular tonus and vigorous shivering movements appear as the body temperature rises, and bodily activity with occasional convulsive shakings supervene later. The North American marmot, on recovery from an artificially induced state which simulates hibernation, has been frequently observed by the writer (see also Dubois, 1896) to curl itself into a compact mass so as to expose the least body surface, and thereupon to exhibit a remarkable erection of the hairs over the whole dorsal aspect. During recovery to normal from very low body temperatures, i.e., 5° to 10°C., this condition is sometimes manifest for several hours. A further noteworthy observation which has been made by many authors (see Dubois, 1896; Voit, 1878) is that the reserve glycogen which is present in the liver in large amounts towards the end of the hibernating period begins to disappear very rapidly in the initial stages of the awakening process. Dubois also noted that during the profound torpor of winter sleep only traces of sugar are present in the blood, and that on resumption of the homoiothermic state the glycemia again reaches the normal limits. Recent evidence (Dworkin and Finney, 1927) shows that during induced hibernation in marmots the blood sugar likewise remains greatly reduced, and that with the appearance of signs of recovery it persistently rises again.

It is very significant that practically all the foregoing reactions, which are associated with the process of awakening from winter sleep, are indicative of increased sympathico-adrenal activities. Furthermore, the phenomena which are related to the process of transition from the homoiothermic to the poikilothermic state suggest strongly the inception of a period of relative inactivity of the sympathico-adrenal mechanism. In this connection, it appears to be well accredited that the normal resistance

to cold is considerably reduced in the absence of adrenal secretion (Lewis, 1923; Marval, 1926; Gans and Miley, 1927). In the experiments on artificial hibernation by Dworkin and Finney the results achieved may have been due to a temporary overwhelming of medulliadrenal function by the large doses of insulin and to the very low temperatures which were employed. Marked evidences of recovery quickly appeared, suggestive indeed of effectual adrenin discharge, when insulin was discontinued for a short space.

The possibility that extraordinary amounts of secreted insulin might serve under natural conditions to initiate hibernation seems scarcely to be tenable. On the other hand, autumnal depression or possibly withdrawal of the natural excitatory stimulus provided by adrenin secretion would tend towards conservation of the organism—apparently poorly-equipped and thermotactically infantile in hibernating types—in the face of climatic rigors and shortage of food. Moreover, a very potent discharge from the adrenal medulla, such as is now definitely known to occur under various conditions of stress (Cannon and Britton, 1927), together with sympathetic impulses, would evidently lead—through such reinforcing effects as those on the heart and circulation, the stimulation of glycogenolysis and elevation of the blood sugar, and the promotion of shivering and increase of body temperature—to abolition of the dormant state and reëstablishment of normal bodily activities. That the recovery of hibernating animals from torpidity to the active state should speedily occur (Dubois, 1896) in case the surrounding temperature falls to a low level (about 0°)—i.e., when a dangerous emergency situation would be created—is in itself highly suggestive and typical of medulliadrenal intervention.

Artificially cooled, hypoglycemic animals are shown by the present results to be severely handicapped in the absence of actively secreting adreno-medullary tissues: they are unable to regain the normal blood-sugar level, they do not shiver, and they evince no tendency to recover their previous body temperature. Animals possessing active adrenal glands manifest, in contrast, a marked ability to recover from the dual condition of severe hypoglycemia and very low body temperature. The experiments accordingly sustain the conception developed in the foregoing paragraphs that hibernation may in great measure be referable to seasonal and economy-serving fluctuations in activity of the medulliadrenal tissues.

SUMMARY

Cats with the adrenal glands intact, which have been rendered hypoglycemic to the convulsive level by insulin, lose heat rapidly on exposure to cold. While the body temperature is falling the skeletal muscles show marked hypertonus, and shivering appears. Coincidently the blood sugar rises to the normal or a hyperglycemic level, and convulsions are

abolished. On withdrawal of the animal from its cold surroundings, evidence of recovery from a very low body temperature (*circa* 25°) is apparent.

Animals without active adrenal medulla, which have been similarly insulinized and exposed to cold, also undergo a rapid diminution of body temperature; in contrast to the responses of animals with active adrenal glands, however, there occurs no reëstablishment of the normal blood-sugar level (see fig. 1), and shivering does not supervene. Convulsions are nevertheless inhibited when the body temperature reaches 25°, although the blood-sugar level may remain in the vicinity of 30 mgm. or 40 mgm. per cent. Muscle hypertonus may be present, but no significant ability to recover from low body temperatures is observed.

If the blood sugar is within normal limits in adrenalectomized animals exposed to cold, shivering may supervene but the glycemic level is scarcely affected.

The shivering reflex is suppressed in hypoglycemic or in hyperglycemic animals when the body temperature is reduced to 20° to 23°, or when it is maintained for one or two hours at somewhat higher temperatures.

Concurrently with the increase in blood sugar which is effected by adrenalin injection in such animals the shivering reflex reappears, the body temperature tends to rise towards normal and recovery appears imminent. Glucose solution also reproduces shivering movements in cooled animals.

Evidence is reviewed and reasons are presented which lead to the belief that a natural autumnal diminution in sympathico-adrenal activity may result, in relatively unstable hibernating organisms, in the commencement of winter sleep. Furthermore, the phenomena which are associated with the recovery of animals from hibernation, and also of artificially cooled homoiothermic animals from low body temperatures, definitely point to increased activity of the sympathico-adrenal mechanism as the immediate agency in the restoration.

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THE THYROID GLAND AND THE SENSITIVITY OF ANIMALS TO INSULIN

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An important relationship to carbohydrate metabolism has at various times been attributed to most of the glands of internal secretion. More particularly the adrenal medulla, the thyroid gland, and within recent months the posterior lobe of the pituitary have been mentioned as significant factors. Many difficulties have admittedly beset investigations into the intimate nature of endocrine activities, however, and a great deal of the evidence which has been brought forward is still lacking confirmatory support.

From many sources in the past few years there has been contributed what may at first glance appear fairly definite proof that thyroidectomy produces a heightened sensitivity to the hypoglycemic effects of insulin. A wide variety of animals has been considered. In 1923 Bodansky compared the effects of insulin on normal and on thyroidectomized sheep and found that the latter suffered a more intense lowering of the blood sugar, and that the time taken to recover from hypoglycemia was also strikingly prolonged. The thyroid gland was, therefore, suggested as being essential to normal recovery from the severe effects of insulin. During the following year similar findings were reported by Houssay and Busso, who used cats, rabbits, guinea pigs and rats, and by Ducheneau, who used rabbits. It is noteworthy that the former workers gave calcium to their animals in order to offset parathyroid deficiency. Later (1925) Burn and Marks showed that rabbits which are deprived of the thyroid gland are hypersensitive to insulin, and furthermore do not give the normal hyperglycemic response to adrenalin (see also Abderhalden and Gellhorn, 1925).

Thyroidectomized dogs have been stated by Houssay and Cisneros (1925) to be more sensitive to insulin than normal controls. Several cases in which the operated animals developed tetany were, however, considered by these authors as positive. Aszodi and Ernst (1927), who also used dogs in their work carried out at the Toronto laboratories, have furnished additional evidence indicating that the function of the thyroid gland is associated with carbohydrate metabolism.

A marked diminution or even disappearance of the glycosuria of experimental diabetes in dogs has been reported by Friedman and Gottesman (1922) to follow ligation of the thyroid arteries or thyroparathyroidectomy. It was suggested by these workers that complete thyroid excision as a remedial measure be tried in human diabetic subjects. Recent evidence (Wolfson, 1927) has shown, however, that no lowering of the blood sugar takes place in pancreatectomized animals after thyroidectomy; moreover, the urinary sugar excretion in such cases may be only slightly diminished. The moribund condition of the animals following the second operation would scarcely allow significant deduction, it appears, from the foregoing experiments.

Thyroxin has been stated by Bodansky (1924) to modify the blood-sugar curve which is observed following insulin administration to sheep.

It is interesting to note that in many different species of animals there is said to occur (Aron, 1926) a striking histological correspondence between the development of the thyroid apparatus and that of the pancreatic islets.

The presence in the body of an important thyroid-insulin interrelationship is strongly suggested by the above concordant evidence of many investigators. Since in some instances, however, the parathyroid glands were either removed or severely traumatized by the experimenters, and also because of the fact that adequate controls on the same animals were not carried out by any of the workers, the conclusions drawn from the experiments cannot in their entirety be acceptable. Excision of the parathyroids alone, it should be stated, may possibly result in a marked increase in sensitivity to insulin (Magenta, 1926). Also, individual variations in the response to insulin of various animals, and of the same animals at different times, have been pointed out on numerous occasions. The observers cited above did not, moreover, pursue their investigations beyond a week or so after removal of the thyroid gland, and the lowered resistance to insulin may have been referable to the general depression incident to anesthesia and major operative procedures. The persistence of the altered response to insulin administration was not established. These considerations, together with the suggestive results of some recent work on the pituitary and adrenal glands (Geiling, Campbell and Ishikawa, 1927; Britton and Geiling, 1927), have led us to make further inquiry into the possibility of a thyroid-insulin interrelationship.

In our studies we have used selected, vigorous cats. Such animals have been found to give fairly uniform reactions to insulin when used over long periods. They were fed on a bread, milk and meat diet, and previous to use in an experiment were kept fasting for a period of 24 hours. The peripheral ear vein was used for drawing off the blood samples, and determinations of blood sugar were made by the Benedict method (1926).

TABLE 1

Experiments showing the reactions of cats to the administration of insulin before and after complete thyroidectomy

CAT NUMBER AND DATE	CAT WEIGHT	INSULIN GIVEN (UNITS PER KILO)	INITIAL (FASTING) BLOOD SUGAR	BLOOD SUGAR 45 MINUTES AFTER INSULIN GIVEN	LOWEST BLOOD SUGAR	TIME AFTER INSULIN OF LOWEST BLOOD SUGAR	GENERAL REACTION
	kilos					*hrs. min.*	
(1)							
1/22/27	4.10	2.00					Absent
1/29/27	3.92	3.00	77	45	27	3 30	Convulsions
2/ 8/27	3.46	2.50	78	52	41	2 15	Slight
2/10/27							(*Thyroidectomy*)
2/17/27	3.35	1.25	70	56	35	2 15	Moderate
2/22/27	3.68	1.50	78	45	41	2 15	Slight
3/ 3/27	3.98	2.50	77	51	30	2 15	Slight
3/15/27	4.28	3.00	71	56	41	3 55	Slight
4/28/27	3.67	3.50	75	46	28	2 05	Convulsions
(2)							
1/22/27	4.00	3.00					Slight
1/29/27	3.90	4.00,	78	46	30	2 00	Convulsions
2/ 3/27	3.75	3.50					Severe
2/ 8/27	3.73	3.50	76		39	2 15	Convulsions
2/10/27							(*Thyroidectomy*)
2/17/27	3.58	1.75	74	59	27	2 15	Convulsions
2/22/27	3.82	1.50	72	52	33	2 15	Slight
3/ 3/27	3.72	3.00	72	66	30	3 45	Severe
3/15/27	4.14	3.50	76	65	32	3 50	Moderate
4/ 7/27	4.13	4.00	91	71	30	2 15	Convulsions
(3)							
1/27/27	2.61	2.00	81	42	41	3 30	Slight
2/ 8/27	2.75	2.50	80	60	46	4 00	Absent
2/10/27							(*Thyroidectomy*)
2/17/27	2.70	1.25	80	47	41	2 15	Slight
2/22/27	3.00	1.50	75	48	35	2 15	Slight
2/26/27	3.12	1.50	70	62	37	2 15	Slight
3/ 3/27	3.03	2.50	61	47	38	3 45	Slight
3/15/27	3.15	3.00	69	55	35	3 50	Slight
4/28/27	3.63	3.50	75	56	30	3 00	Moderate
5/12/27	3.90	4.00	76	50	20	4 00	Convulsions
(6)							
2/24/27	3.84	3.00	66	33	25	2 50	Convulsions
3/17/27		2.50	66	47	30	3 55	Moderate
3/22/27							(*Thyroidectomy*)
3/29/27	3.66	1.50	60	45	45	0 45	Slight
4/ 7/27	3.83	1.75	75	50	35	2 20	Severe
4/26/27	4.30	3.00	80	55	30	2 30	Convulsions
5/ 3/27	4.40	2.50	70	45	30	2 30	Severe
5/25/27	4.27	2.50					Slight

Preliminary control observations on the response to insulin were made at intervals over a period of 2 to 4 weeks on each animal before carrying out the thyroidectomy operation, and the individual variations in response which provided data for comparison were thereby established. The technique suggested by Dott (1923) was followed, with slight modifications, in making the complete excision of the thyroid gland with least disturbance of the parathyroid bodies. By careful dissection it was found possible to leave usually three and in one case four of the parathyroids intact. Rapid recovery occurred from the operation, and a week or so later and at intervals thereafter the responses to insulin were again determined.

The observable general reactions of the animals to insulin have been noted under different headings, i.e., slight, moderate, severe and convulsive, representative of different degrees of severity of the reaction (see Britton and Geiling, 1927).

RESULTS. The results of our major experiments are given in brief in table 1. It will be observed that during the preliminary control periods the injection of from 1 to 3 units of insulin per kilo weight was generally borne without severe reaction, although the latter dosage sometimes produced convulsive seizures.

Cat 1 suffered no apparent general effects, before the thyroid gland was excised, from 2 units of insulin per kilo, and only a slight hypoglycemic reaction from 2.5 units. During the latter experiment the lowest blood sugar recorded was 41 mgm. per 100 cc., determined 2¼ hours after insulin was given. Seven days after thyroidectomy, however, the blood sugar fell to 35 mgm. in 2¼ hours following the injection of only half the dosage of insulin (1.25 u.p.k.); moreover, the general reaction was correspondingly more pronounced. On March 3 (21 days after operation) the lowest blood sugar produced by 2.5 u.p.k. of insulin was 30 mgm. per 100 cc., although the general symptoms, as with the same dose of insulin before operation, were merely slight. It is evident that by March 15 complete recovery of the normal response to insulin was established, since on this date 3 u. p. k. failed to lower the blood sugar below 41 mgm., and only a slight reaction was observed. At this time, indeed, the animal may be said to have withstood the injection of insulin much better than before operation: for example, on January 29 a dose of 3 units produced the very low blood sugar of 27 mgm. per cent, and coincidently, severe convulsions. The post-operative convulsive dose in cat 1 was observed to be 3.5 units per kilo.

The record of cat 2 likewise agreed with the above in showing an increased sensitivity to insulin following excision of the thyroid gland. Furthermore, it was again noted that the change was merely a transient one. Under normal conditions, with the thyroid gland intact, 3.5 units of insulin per kilo proved to be a convulsive dose; one week after operation, i.e., on February 17, half this amount of insulin brought about convulsions, whereas

in less than four weeks after the latter date 3.5 units produced but a moderate reaction.

In complete accord are the responses shown in the succeeding cases of table 1. Cat 3 showed a diminished resistance to insulin for a brief period after thyroid removal; 21 days after operation its responses had become normal, and as time passed it began to show a heightened tolerance towards insulin action. Similar behavior was also displayed by animal 6: a slightly longer period—from four to five weeks—elapsed in this case before recovery occurred from the lowered (post-thyroidectomy) resistance to insulin.

It is noteworthy that the general condition of the animals following removal of the thyroid remained apparently good. Food was taken readily within two days after operation, and decided increases in weight were eventually shown in all instances: Cats 1 and 2 gained 930 and 560 grams respectively in 26 days; no. 3 gained 1200 grams (an increase of 44 per cent) in 12 weeks; and no. 6 gained 740 grams in 5 weeks (see table 1). These animals were evidently very fat—an observation that was confirmed at autopsy. The insulin dosage per unit of *active* body tissues a few weeks after thyroidectomy was, therefore, somewhat higher than the figures actually denote. None of the operated animals showed signs of tetany or increased irritability; in fact, they became slightly more lethargic than the normal controls. Several days were allowed for recovery, it will be observed, after each experiment in which insulin was used.

Consideration of a larger series of cases, as at first proposed, was rendered unjustifiable in view of the consistency of the results. Each experimental animal served, it is apparent, as its own control. Nevertheless, other control animals were also studied. In two instances dummy cervical operations were performed in which a similar technique was employed without interference with the thyroid gland, and the comparison of responses to insulin was made both before and subsequent to these procedures. Contrasted with the results following thyroidectomy, these animals showed no fluctuations in response beyond those slight variations usually obtained from normal animals. In a number of other control (normal) animals, insulin was administered at 7 to 10-day intervals over periods of several months. These cases also showed no significant variation, as time passed, from the usual hypoglycemic response.

The slight and transient changes in the reaction to insulin of thyroidectomized animals may possibly be due either to a general reduction of metabolic activity, or to specific depressor effects on the sympathetic or the adrenal mechanism. Our experiments are being continued but at present they offer no direct evidence on these points.

Effect of post-pituitary extract on hypoglycemic, thyroidectomized animals. The possibility of diminution of the glycogen reserves in thyroidecto-

mized, insulin-hypersensitive animals has been inquired into by several methods. In a short series of experiments on such animals the administration of small amounts of posterior pituitary extract (U. S. P.) during severe hypoglycemia did not fail to bring about a rapid increase in blood sugar and amelioration of the convulsive seizures. Two cases are cited below.

Cat 2. February 10, 1927, thyroidectomy. February 17, 0 hour, blood sugar 74 mgm. per cent, 1.75 units per kilo of insulin injected; at 3 hours, 10 minutes, severe prostration, convulsions; 3 hours, 20 minutes, blood sugar 33 mgm., 1.0 cc. pituitary extract (U. S. P.) given subcutaneously; 3 hours, 25 minutes, cat walked across room; 3 hours, 30 minutes, blood sugar 68 mgm., cat appeared normal.

Cat 6. March 22, 1927, thyroidectomy. April 7, at 0 hour, blood sugar 75 mgm. per 100 cc., 1.75 units per kilo of insulin given; at 3 hours 5 minutes, blood sugar 40 mgm., marked weakness, convulsions; 3 hours 6 minutes, 0.5 cc. pituitary extract injected subcutaneously; 3 hours 17 minutes, cat walked about room, appeared normal; 3 hours 20 minutes, blood sugar 60 mgm.

The very rapid elevation of the blood-sugar level in these cases precluded the possibility of its formation from either protein or fat. It was indicated, therefore, that the hepatic tissues of animals deprived of the thyroid gland contained considerable amounts of glycogen.

Thyroidectomy and adrenalin hyperglycemia. The effects on the blood sugar percentage of the administration of adrenalin hydrochloride were also tested in three thyroidectomized animals. Injections were given by the intraperitoneal route. In each case a fasting period of 24 hours was observed before the experiment. Protocols derived from a few cases are given in brief below.

Cat 1. February 10, 1927, complete thyroidectomy. April 21, fasting blood sugar 90 mgm. per 100 cc.; adrenalin injected, zero time, 0.2 mgm. per kilo; 15 minutes, blood sugar 113; 1 hour, 100; 2 hours, 81. April 26, initial blood sugar 78 mgm. adrenalin, 0 2 mgm. per kilo; 20 minutes after adrenalin given, blood sugar 93 mgm.; 30 minutes, 101; 1 hour, 90; 2 hours, 85.

Cat 3. February 10, 1927, thyroid gland removed. April 12, fasting blood sugar, 80 mgm. per cent; adrenalin 0.2 mgm. per kilo at 0 minute; 15 minute, blood sugar 105; 30 minutes, 118; 1 hour, 128; 2 hours, 117. April 21 initial blood sugar 74 mgm.; adrenalin 0.2 mgm. per kilo; 20 minute after adrenalin, blood sugar 84 mgm.; 30 minutes 110; 1 hour, 113; 2 hours, 94. April 26, fasting blood sugar 75 mgm.; at 0 minute, adrenalin 0.2 mgm. per kilo; 20 minutes, blood sugar 91; 35 minutes, 110; 1 hour, 113; 2 hours, 94.

The evidence again suggests, therefore, the probability that glycogen storage occurs in the absence of the thyroid gland.

Medulliadrenal inactivation after thyroidectomy. The adrenal medulla in dogs may still secrete normally for at least a few days after thyroparathyroidectomy, according to Czarnecki and Sarabia (1927). In the majority of a group of cases reported by Sun (1927), the amount of adrenin

content per gram of adrenal substance was increased in rats which were thyroparathyroidectomized at 100 days of age and killed 50 days later.

In one case we have examined the effect of insulin action on the thyroidless animal after elimination of the adrenal medulla. The operation was carried out on cat 1 on May 6, 1927, nearly three months after excision of the thyroid gland, when the normal resistance to insulin had been fully reëstablished. The post-operative condition of the animal remained excellent. It will be observed (table 1) that on March 15 it had shown only a slight reaction to 3 units of insulin per kilo; on April 28 convulsions were produced when 3.5 units were administered. Six days after removal (by incision of two poles of the gland and through suction) of the adrenal medulla, i.e., on May 12, the animal sustained severe convulsions 90 minutes after injecting only one unit of insulin per kilo. Frequent injections of glucose were given (altogether, 50 cc. of a 20 per cent solution by the intraperitoneal route) before the animal showed signs of recovering, over four hours later, from convulsive seizures. On May 19, and again on May 25, convulsions were produced by one-half unit of insulin per kilo, and on May 27 severe convulsions occurred within two hours after the administration of only three-tenths of a unit per kilo.

A very marked increase in sensitivity to insulin persisted in the above instance without diminution, therefore, for three weeks after eliminating the adrenal medulla. The observation is in striking contrast to the slight and transient change in response to insulin produced in the same animal, as well as in others of the series, by thyroid ablation alone.

It was noted further that a small amount of adrenalin given to the thyromedulliadrenalectomized animal during hypoglycemic convulsions rapidly brought about amelioration of the symptoms.

Further experiments related to the adrenal mechanism and insulin action are reported elsewhere (Britton and Geiling, 1927).

Glycogen content of tissues and results of necropsy. Three of the animals which were used in the foregoing experiments (nos. 1, 3 and 6) were guillotined on May 27, and analyses of the liver and muscles were made for glycogen by Pflüger's method. The results were as follows: Cat 1 (fasting 24 hours, killed during severe insulin convulsions), liver glycogen 3.96 per cent, muscle glycogen 1.02 per cent; cat 3 (fasting 24 hours), liver 3.48, muscle 1.10 per cent; cat 6 (fasting 24 hours), liver 3.96, muscle 0.80 per cent.

Careful search for traces of thyroid tissue was made by macroscopic dissection at necropsy in the cases of cats 1, 3 and 6. This proved negative. Cat 2, after death from a respiratory infection, was incinerated before an examination could be carried out.

SUMMARY

The reactions of animals to insulin before and after complete thyroidectomy (with preservation of the parathyroid bodies) have been studied over periods of several months. A definite increase in sensitivity to insulin, not referable to operative trauma, is observed for 10 to 20 days after the operation. Approximately one-half of the pre-operative reactive dose of insulin is sufficient to produce hypoglycemic symptoms during this period. The hypersensitivity may be incident to a general reduction of metabolic activity, or to a temporary depression of the sympathico-adrenal mechanism.

Complete recovery of the normal resistance to insulin occurs in 20 to 30 days after thyroid removal.

Within five or six weeks after thyroidectomy a heightened tolerance towards insulin is established.

During the temporary post-operative period of increased sensitivity to insulin in thyroidless animals, posterior pituitary extract (U. S. P.) is very effective in elevating the blood sugar from the hypoglycemic to a normal level and abolishing convulsions.

Typical adrenalin hyperglycemia may be readily induced in thyroidectomized cats.

Ablation of the adrenal medulla in an already thyroidectomized animal resulted in an extreme and persistent reduction of the normal ability to withstand insulin. The injection of adrenalin in this case during severe insulin shock rapidly brought about recovery.

The liver and muscle of fasting thyroidectomized animals, and also of one thyromedulliadrenalectomized animal which was killed during hypoglycemic convulsions, contained normal amounts of glycogen.

No evidence was forthcoming at autopsy of remnants or regeneration of the thyroid gland.

The results indicate that the operation of thyroidectomy, and also of thyromedulliadrenalectomy, is not followed by any noteworthy depression in glycogen storage. In emergency situations such as created by the injection of insulin, however, there is observed a reduced ability to mobilize carbohydrate reserves. Following thyroid removal the disability is slight and temporary and possibly referable to interrelated reactions, whereas after elimination of the adrenal medulla it is severe and permanent and apparently due specifically to suppression of adrenin secretion.

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MEDULLIADRENAL SECRETION AND CARBOHYDRATE METABOLISM

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Much vigorous discussion has taken place during the past decade with reference to the physiology of the adrenal glands. One phase of the discussion has been concerned with the relationship of the adrenals—particularly the medulla—to carbohydrate metabolism. While some workers have forcefully asserted that the medullary secretion exerts no important or indispensable function in this connection, others have presented evidence which indicates that a relationship does exist and is of indubitable importance.

The action of insulin on rabbits after complete adrenalectomy does not differ noticeably from its action on normal rabbits, according to evidence brought forward by Stewart and Rogoff (1923a). These workers used three animals on which the adrenal operations had been carried out from four weeks to over eight months previously, and one control animal. Various doses of insulin were given, and in one case an animal showed unaccountably strange symptoms following an injection of the material they used. Furthermore, the reactions to insulin were not ascertained before operation, and in view of the great variability among rabbits in response to the same dosage of insulin, it does not appear that significant deductions can be drawn from the above few cases. From another series of experiments in which the genesis of diabetes in dogs after pancreatectomy was considered, the same authors (1923b) concluded that there was no basis for theories assigning to the adrenals a special relationship to the pancreas in the regulation of carbohydrate metabolism and the blood-sugar content. In a review of the subject by Stewart (1924) in which the foregoing line of evidence was vigorously defended, adrenin secretion is adjudged as unimportant and its suppression without influence on the life of the organism.

Results which have been reported from other laboratories are not in agreement with the experiments and conclusions of Stewart and Rogoff. In 1923 Sundberg found that rabbits deprived of the adrenal glands offered less resistance to insulin than normal controls. The animals were examined within a few days after operation, however, and a very high

mortality rate was reported. Lewis (1923) also observed that the lethal dose of insulin for adrenalectomized rats was much smaller than that for another group of animals with the adrenal glands intact.

In transfusion experiments on suprarenal-jugular anastomosed dogs, Houssay, Lewis and Molinelli (1924) noted that during insulin hypoglycemia in the donor the blood sugar in the recipient was definitely increased —in one case from 64 mgm. to 300 mgm. per cent. It was concluded, therefore, that secretion from the adrenal medulla is markedly stimulated on lowering the blood sugar by insulin.

Furthermore it has been shown by Cannon, McIver and Bliss (1924) that in cats an accelerated secretion from the adrenal medulla occurs in company with a general discharge of sympathetic nerve impulses when the blood sugar is reduced by insulin. In animals without active adrenal glands the fall of blood sugar was greater and the convulsive seizures were induced sooner and with smaller doses of insulin than in those animals in which the adrenals were present.

Two bilaterally adrenalectomized dogs which were observed by Hallion and Gayet (1925) withstood insulin very poorly; since the animals were injected only three hours after the operation, however, the results can hardly be considered significant.

Marked sensitivity of rats to insulin has been found by Artundo (1927) to be present for a period of two weeks after removal of the adrenal glands; a few weeks later this sensitivity is said to be greatly diminished. Somewhat similar fluctuations in the resistance of adrenalectomized rats to various toxic substances have been described by many authors (Belding and Wyman, 1926; Lewis and Torino, 1925, 1927; Crivellari, 1927). In disagreement are the findings of Stewart and Rogoff (1922) and of Rogoff and DeNecker (1925), who used morphine as a test on their animals.

Various changes in the adrenal glands of dogs and rabbits have been stated to follow the administration of insulin (Riddle, Honeywell and Fisher, 1924; Kahn, 1926). According to Thatcher (1926), however, insulin given to rabbits over periods up to eight months does not cause any gross hypertrophy of the adrenal tissues.

In the greater portion of the work referred to above the adrenal glands (cortex and medulla) have been considered as a whole, and complete excision of both glands has been carried out. It is nevertheless well known that most animals which have undergone bilateral adrenalectomy quickly become moribund; such animals cannot, obviously, be considered as significant physiological indicators. In this connection may be mentioned a timely critique on the use of recently operated animals as test objects which has been passed by Rogoff and DeNecker (1925). In the foregoing work, moreover, nearly all the experiments have compared one series of operated animals with another series of unoperated and (presumably) normal con-

trols. While a majority of the workers agree that the adrenal glands constitute an important factor in relation to carbohydrate metabolism, the evidence is nevertheless conflicting, and is in some instances founded on two or three experiments. These are among the considerations which have led us to make further inquiry into the subject.

METHODS. Cats were used in the experiments. Insulin was administered after a 24-hour fasting period, and the analyses for blood sugar were made by the method of Hagedorn and Jensen (1923), the peripheral ear vein blood being used. All the blood-sugar determinations were carried out by one of us (G.) in one laboratory, without any knowledge at the time of the nature of the experiment being undertaken by the other workers in another laboratory. Pflüger's method was used for making the glycogen analyses.

The adrenal medulla was inactivated in a majority of the cases by excision of the right and denervation of the left gland. The left splanchnic nerves were cut and from 1 to 2 cm. of the upper left lumbo-abdominal sympathetic trunk were excised in carrying out the denervation. In some instances the adrenal medulla was evacuated by incising the upper and lower poles of the gland and applying thorough suction. Each method of inactivation subsequently was found to yield similar experimental results. Dummy and other control operations, described in detail later, were also performed. The responses to insulin (from one batch) were determined both before and after operation. Several days (6 to 8) were usually allowed for recovery from the operative trauma; a few of the animals were tested shortly after operation, and only 3 or 4 days were allowed in these cases. The results obtained even four or five months after operation did not differ noticeably, it may be mentioned, from the earliest observations.

It was found desirable and convenient to classify the reactions occurring during insulin hypoglycemia according to their degree of severity. They are thus noted as "slight," when the animal showed a condition of heightened excitability and a swaying or stumbling gait on attempting to walk; as "moderate," when the hind limbs manifested increasing weakness and a few steps only could be taken without difficulty and resting periods; as "severe," when paresis had spread to the fore limbs and the animal was prostrated, respiration was rapid, the pupils were widely dilated, salivation was profuse, and (frequently) the external sphincters were relaxed. In those instances in which convulsions supervened, these typical reactions were merely transitional phases.

RESULTS. Comparison may be made of the reactions of animals to insulin administration before and after medulliadrenal inactivation by reference to tables 1 and 2. At a glance it will be observed that the operation produced a remarkably sensitized animal: commonly the blood

TABLE 1

Control experiments showing the reaction of normal cats to insulin administration

Compare with table 2

CAT NUMBER AND DATE	CAT WEIGHT	INSULIN (UNITS PER KILO)	BLOOD SUGAR					HYPOGLYCEMIC REACTION
			Initial, 0 minute	45 minutes	2 hours	3½ hours	5 hours	
	kilos		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	
(*22*)								
12/16/26	4.4	2.0						Absent
12/22/26	4.5	2.0	93	56	32	70	73	Absent
(*23*)								
12/21/26	3.4	2.0	81	41	41	43	50	Severe
12/29/26	3.5	2.0	72	47	43	43	48	Severe
(*24*)								
12/21/26	2.7	2.0	79	57	43	50	59	Moderate
12/29/26	2.7	2.0	79	51	48	51	51	Slight
(*25*)								
12/23/26	3.1	2.0	75	33		43	71	Slight
12/30/26	3.2	2.0	88	53	40	45	67	Moderate
1/28/27	3.7	4.0	89	58				Moderate
(*26*)								
12/23/26	2.9	2.0	86		43	43	49	Absent
12/30/26	2.9	2.0	92	54	45	45	45	Absent
(*27*)								
1/19/27	3.2	2.0	88	49	40	31	34	Moderate
2/ 7/27	3.4	2.0	98	70	34	38		Moderate
2/28/27	3.5	0.5	81		37	79		Absent
(*28*)								
1/19/27	2.8	2.0	79	47	39	34	68	Absent
2/ 7/27	2.8	3.0	96	47	36	34		Convulsions at 165 minutes
(*29*)								
1/21/27	3.1	1.0	83	36	49	61	90	Severe
2/14/27	3.1	0.3	80	38		60		Absent
(*30*)								
1/21/27	2.8	3.0	82	38	47	65	59	Absent
2/ 2/27	2.8	4.0	83	61	40	30		Slight
2/14/27	2.7	2.0	80	51	47	36		Absent
(*34*)								
2/25/27	2.8	2.0	81		39			Absent
3/ 7/27	2.7	3.0	84	33	33			Absent
3/21/27	2.9	4.0	77		16			Convulsions at 180 minutes

TABLE 2

Experiments showing the reaction to insulin of cats with the adrenal medulla inactivated

Compare with table 1

CAT NUMBER AND DATE	CAT WEIGHT	INSULIN (UNITS PER KILO)	BLOOD SUGAR			HYPOGLYCEMIC REACTION
			Initial, 0 minute	45 minutes	2 hours	
	kilos		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	
(22)						
1/ 7/27						*(Adrenals inactivated)*
1/14/27	4.3	1.0	93	63	33	Severe
1/19/27	4.3	2.0	79	56	34	Severe
3/31/27	3.7	2.0	85			Convulsions at 170 minutes
(23)						
1/ 8/27						*(Adrenals inactivated)*
1/14/27	3.6	1.0	88	61		Convulsions at 90 minutes
1/19/27	3.9	0.5	78	59	27	Severe
2/ 2/27	3.8	1.0	86	75	37	Severe
2/ 7/27	3.7	1.0	89	72	36	Severe
2/18/27	3.7	1.0	96		24	Convulsions at 120 minutes
3/31/27	3.7	1.0	85		38	Convulsions at 75 minutes
4/ 8/27	3.7	0.5	97		23	Moderate
5/11/27	3.7	1.0	105		37	Moderate
(24)						
1/10/27						*(Adrenals inactivated)*
1/17/27	2.6	1.0	91	42		Convulsions at 75 minutes
1/26/27	2.4	0.5				Convulsions at 85 minutes
2/ 4/27	2.4	0.3	82	82	32	Slight
2/18/27	2.2	1.0	87		26	Convulsions at 60 minutes
(25)						
2/24/27						*(Adrenals inactivated)*
2/28/27	3.6	1.0	81		32	Convulsions at 90 minutes
3/ 7/27	3.6	0.5	96	42		Slight
5/11/27	3.5	1.0	80		44	Convulsions at 150 minutes
(26)						
1/25/27						*(Adrenals inactivated)*
1/31/27	3.5	1.0	83	77	26	Convulsions at 105 minutes
2/ 4/27	3.5	0.5	81	72	50	Slight
2/18/27	3.5	1.0	87		38	Severe
3/23/27	3.2	1.0	104		44	Severe
5/11/27	3.2	1.0	85		40	Convulsions at 150 minutes
(27)						
3/ 1/27						*(Adrenals inactivated)*
3/ 4/27	3.4	0.5	67		22	Convulsions at 110 minutes
3/16/27	3.3	0.3	91		17	Convulsions at 95 minutes
5/11/27	3.2	0.5	76		29	Convulsions at 80 minutes

TABLE 2—*Concluded*

CAT NUMBER AND DATE	CAT WEIGHT	INSULIN (UNITS PER KILO)	BLOOD SUGAR			HYPOGLYCEMIC REACTION
			Initial, 0 minute	45 minutes	2 hours	
	kilos		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	
(*28*)						
2/24/27						(*Adrenals inactivated*)
2/28/27	2.6	1.0	84		34	Convulsions at 95 minutes
3/ 7/27	2.8	0.5	76		23	Convulsions at 95 minutes
3/14/27	2.7	0.3	73		32	Convulsions at 85 minutes
4/ 8/27	2.6	0.2	70		41	Slight
5/11/27	2.7	0.5	113		26	Convulsions at 80 minutes
(*29*)						
3/ 1/27						(*Adrenals inactivated*)
3/ 4/27	3.2	0.3	92		38	Convulsions at 115 minutes
3/ 9/27	3.2	0.3	75		32	Convulsions at 125 minutes
3/15/27	3.0	0.2	79		39	Convulsions at 140 minutes
4/ 8/27	2.9	0.2	72		37	Convulsions at 135 minutes
(*34*)						
3/24/27						(*Adrenals inactivated*)
4/ 1/27	2.7	1.5	78		29	Convulsions at 110 minutes
4/ 7/27	2.9	1.0	97		32	Convulsions at 110 minutes
5/ 6/27	3.0	0.5	72		29	Severe

sugar fell much lower and the hypoglycemic reaction was much more severe and protracted, although considerably smaller doses of insulin were used.

Before operation, for example, cat 24 underwent at one time a moderate and at another time a slight reaction following the injection of 2 units of insulin per kilo body weight. The lowest blood sugar recorded during the experiment was 43 mgm. per cent. After the adrenal medulla had been rendered inactive, however, convulsions took place following 0.5 u. p. k. of insulin, and in another instance with only 0.3 unit the blood sugar fell as low as 32 mgm. per cent. Again, no. 27 had shown only a moderate reaction to 2 units of insulin when the adrenal glands were intact, whereas, fifteen days after medulliadrenal inactivation, 0.3 unit brought about convulsions and the extremely low blood-sugar level of 17 mgm. per cent in 95 minutes. In an experiment on cat 29, which also possessed no active adrenal medulla at the time, convulsions occurred following the minute dose of 0.2 u. p. k. of insulin.

The responses of no. 28 offer very significant evidence. Before operation it was necessary to inject 3 units of insulin per kilo in order to produce convulsions, and these did not begin until 165 minutes after the injection.

After removing the adrenal medulla, however, it was found that only one-tenth of the pre-operative convulsive dose, i.e., 0.3 unit per kilo, precipitated severe convulsions in approximately half the previous time (85 minutes). In the latter case, also, the blood sugar during insulin action fell to a lower level. Before operation in another instance the animal showed no general reaction from the injection of 2 u. p. k. of insulin, while over six weeks after operation, hypoglycemic symptoms were produced by 0.2 unit per kilo. These and other typical responses are graphically represented in figure 1.

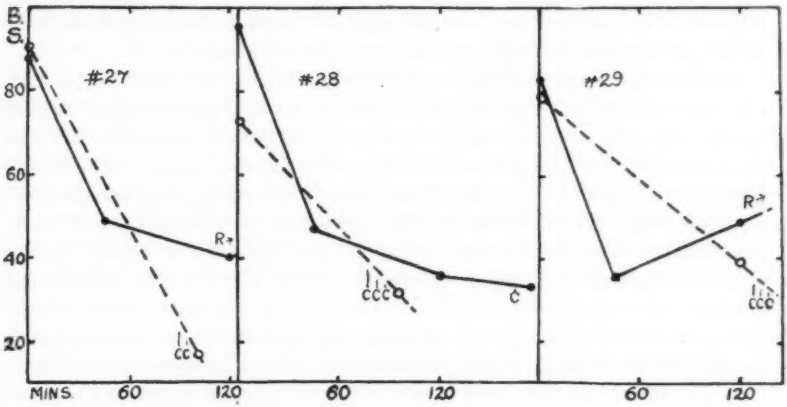

Fig. 1. Curves illustrating the effects of insulin on cats before (continuous lines) and after (dash lines) medulliadrenal inactivation. In all cases, much larger doses of insulin were borne with much less effect before operation. No. 27, with adrenals active, recovered spontaneously after showing only a moderate hypoglycemic reaction from 2 u.p.k. of insulin, whereas after the adrenal operation convulsions were very quickly produced by 0.3 u.p.k. No. 28 similarly underwent convulsions, three weeks after operation, from 0.3 u.p.k. of insulin, although it had been necessary to give ten times that dose in order to provoke convulsions when the adrenals were active. No. 29, before adrenal inactivation, made good recovery from 1.0 u.p.k., while after operation the minute dose of 0.2 u.p.k. produced severe convulsive seizures. Insulin given at 0 minute; C = convulsions; R = recovery.

Analysis of the cases cited in tables 1 and 2 shows that in 24 experiments which were carried out on animals with adrenals active, in only two instances did convulsions occur (at 165 minutes and at 180 minutes after insulin was given), while reactions were absent in 11 cases. The average dose of insulin injected was slightly over 2 units per kilo, and the blood sugar fell, in the average of all experiments, to 42 mgm. per cent at the end of the two-hour period. In the majority of cases the recovery from hypoglycemia was spontaneous.

In marked contrast to the foregoing is the observation that, subsequent to removal of the adrenal medulla, in no instance in a total of 38 experiments was the hypoglycemic reaction absent. In 24 experiments convulsions occurred—in 12 cases in less than 100 minutes, and in 6 cases in 100 to 120 minutes after insulin—and artificial aid was necessary in all these experiments in order to bring about recovery to normal. Furthermore, the average dose of insulin to medulliadrenal inactive animals was only 0.78 unit per kilo. At the two-hour period the blood sugar (all cases averaged) was 32 mgm. per cent.

The initial blood-sugar values, observed in each case subsequent to removal of the adrenal medulla and after a 24-hour fasting period, show no significant change from the normal (pre-operative) levels.

In addition to the foregoing series of individually controlled cases, various other control experiments were carried out. Dummy operations, in which approximately the same amount of intra-abdominal trauma was committed without disturbance of the adrenals, resulted in two cases in negative findings: there occurred only a slight lowering of the resistance to insulin during the week subsequent to operation. Section of the left splanchnic nerves in another animal resulted in no appreciable change in the response to insulin. A slight increase in sensitivity to insulin supervened, however, on excision of the right adrenal gland in a further control experiment. No striking changes in response were observed in a number of animals which were kept for long periods (two or three months) under laboratory conditions and subjected once in eight or ten days to reactive doses of insulin; in some cases a slightly increased tolerance towards the pancreatic hormone was evident.

The suppression of sympathetic activity in normal and in medulliadrenal inactive animals was attempted, in a few cases, by the administration of ergotamine. The effect of insulin action on these animals was then observed. Noteworthy differences in response to insulin occurred in those cases in which the adrenal glands were intact, after the intraperitoneal injection of 0.3 mgm. of ergotamine tartrate per kilo: the blood sugar fell lower than in the previous control experiments and the general symptoms were more marked. Similarly it was found that the sensitivity to insulin of animals without active adrenal medulla was more pronounced subsequent to the injection of ergotamine. In accord with the evidence brought forward by Cannon, McIver and Bliss, the results suggest that sympathetic activity coöperates with the adrenal mechanism in the resistance set up by the organism to insulin shock.

Concurrent type of convulsions in animals without active adrenal medulla. During severe hypoglycemic prostration in medulliadrenal inactivated animals the convulsive fits commonly manifested a tendency to become concurrent and fluctuant in character. In the later stages of insulin pros-

tration this tendency was particularly evident. General tonico-clonic contractures were also frequently observed between the peak of the convulsions. The following cases are illustrative: Cat 22, May 13, 1927; insulin, 1 unit per kilo, at 10:25 a.m.; convulsions (acme of seizures) at 11:58, 12:03 p.m., 12:06, 12:10, 12:14. Cat 24, February 18; insulin 1 u. p. k., at 9:38 a.m.; convulsions at 11:10, 11:14, 11:15, 11:16, 11:17, 11:21, 11:23. 11:31, 11:36, 11:41, 11:46, 11:49. Cat 26, January 31: insulin, 1 u. p. k., at 9:04 a.m.; convulsions at 11:11, 11:12, 11:14, 11:16, 11:19. Cat 27, April 1; insulin, 1 u. p. k., at 9:50 a.m.; convulsions at 1:16 p.m., 1:20, 1:23, 1:25. In such cases restoration to normal (by artificial means) was usually much protracted.

These results are in marked contrast to those elicited from control animals with the adrenal glands intact. In such cases the occurrence of convulsive seizures (after heavy insulin dosage) was commonly postponed until 3 to 5 hours after insulin injection, and intervals of 30 to 60 minutes between the seizures were often observed. During such intervals the animals sometimes walked about the room and otherwise acted normally. In one case an animal with intact adrenal glands recovered spontaneously after an injection of 4 units of insulin per kilo, and in another instance there were indications of recovery 6½ hours after the administration of 6 u.p.k. of insulin.

Mobilization of glycogen during convulsive seizures. Attention has been drawn by Cannon and his associates (1924) to a mechanism protecting the body from dangerous hypoglycemia which probably operates in two stages —a primary stage in which sympathetic activity with adrenal secretion occurs and mobilizes sugar from the liver, and, if this proves inadequate, a secondary stage in which the activities of the first stage are intensified and augmented in convulsive seizures. As mentioned in the foregoing section, we have observed that animals with the adrenal glands intact may exhibit normal bodily reactions shortly after undergoing convulsions, or between the convulsive fits. Inquiry into the blood sugar changes in these cases revealed interesting data.

Cat 33 (normal) was on March 7 given 1.5 units of insulin and therefrom suffered a slight convulsion; shortly before the convulsion the blood sugar was 37 mgm. per cent; 22 minutes after the convulsion, when the animal appeared normal with the exception of slight weakness, the blood sugar had increased to 44 mgm. The same animal underwent convulsions on March 23 after the injection of 3.5 units of insulin per kilo; before the convulsions (two seizures) the blood sugar was 36 mgm.; at intervals the animal walked about the room; 23 minutes after the second convulsion the blood sugar was 37 mgm. Again, on May 13 cat 36 showed an increase in blood sugar from 30 mgm. to 41 mgm. following convulsions produced by 2 units of insulin per kilo. Later this animal spontaneously recovered to a normal condition.

No such augmentation of the blood-sugar level following convulsive seizures was observed in animals after removal of the adrenal medulla: usually, indeed, a diminution occurred. The glycemic percentage fell from 26 mgm. to 23 mgm. in cat 28 on March 7, for example, after convulsions which were precipitated by the injection of only 0.5 unit of insulin per kilo. Similarly, in no. 29 on March 4 the blood sugar decreased from 38 mgm. to 33 mgm. after administering 0.3 u. p. k. of insulin, and on April 8 from 37 mgm. to 25 mgm. after only 0.2 unit, the lower blood-sugar reading in each case being recorded shortly after convulsions took place. These animals, furthermore, would apparently have succumbed had no steps been taken to promote their recovery.

Medulliadrenal inactivation and the effects of adrenalin. The reactions to adrenalin of normal animals have in a number of cases been compared with those elicited from animals without active medulliadrenal tissues. Very small amounts of adrenalin injected subcutaneously into normal fasting cats were found to affect the blood-sugar level scarcely at all: the readings obtained in the case of no. 33 on March 2 varied merely from 82 mgm. (initial) to 84 mgm., and in no. 37 on March 16 there was no variation from an initial blood sugar of 80 mgm., after 0.2 mgm. of adrenalin per kilo had in each case been administered. The interval between the drawing of the blood samples was 45 minutes. Under similar experimental conditions, except that the adrenal medulla had previously been rendered inactive, the glycemic percentage rose from 86 mgm. to 182 mgm. in cat 22 on February 28, and on the same date in cat 24 from 80 mgm. to 104 mgm. When injected intraperitoneally, 0.1 mgm. of adrenalin per kilo produced in no. 26 (adrenal medulla inactive) on April 6 an increase in blood sugar from 100 mgm. (initial) to 137 mgm. in 30 minutes, whereas in no. 40 (a normal animal), on the same date and under the same conditions, a slight change from 114 mgm. to 123 mgm. occurred. There was observed in medulliadrenal inactivated animals, therefore, a notably greater sensitivity to the injection of small amounts of adrenalin.

Table 3 shows in a striking manner the hypersensitivity to adrenalin of animals in which the secretion of adrenin had been suppressed, in comparison with normal animals. It will be noted that in all cases adrenalin was injected (usually 0.1 mgm. per kilo, subcutaneously) after reduction of the blood sugar by insulin, and often during hypoglycemic convulsions. Restoration to an apparently normal condition was commonly effected a short while after the injection. Those animals which were intractable to the smaller doses of adrenalin were sometimes but not invariably restored by larger amounts.

Particularly noteworthy is the observation that in the case of medulliadrenal inactive animals, very much larger increases in blood sugar usually followed the introduction of adrenalin. In nine experiments the range of

TABLE 3

Experiments showing the effects of adrenalin administered to animals during insulin hypoglycemia

Cases starred (*), adrenal medulla inactive; others, normal

CAT NUMBER AND DATE	INSULIN GIVEN (UNITS PER KILO)	BLOOD SUGAR (1) Initial, 0 minute	BLOOD SUGAR (2) After depression by insulin	BLOOD SUGAR (3) After injection of adrenalin	TIME BETWEEN (1) AND (2) BLOOD SUGARS		TIME BETWEEN (2) AND (3) BLOOD SUGARS		ADRENALIN INJECTED	OBSERVATIONS
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*hrs.*	*min.*	*hrs.*	*min.*	*mgm. per kilo*	
(23)										
*1/28/27	1.0	83	54	112	2	00	1	35	0.1	Normal condition restored
*2/ 2/27	1.0	86	35	137	3	40	1	40	0.1	Rapid recovery
(24)										
*2/ 4/27	0.3	82	80	111	3	30	1	00	0.1	(No hypoglycemic symptoms)
(25)										
1/28/27	4.0	89	62	69	2	00	1	35	0.1	(No hypoglycemic symptoms)
*3/ 7/27	0.5	96	60	87	3	30		40	0.1	Weakness diminished
(26)										
*1/31/27	1.0	83	26		2	00			0.1	No effect on convulsions
*2/ 4/27	0.5	81	30	104	3	30	1	00	0.1	Weakness abolished
(27)										
2/ 7/27	2.0	98	38	87	3	30	1	30	0.1	Moderate weakness abolished
2/11/27	2.0	82	46	40	2	00	1	20	0.1	Convulsions unaffected
2/28/27	0.5	81	79	111	3	45		45	0.1	(No hypoglycemic reaction)
(28)										
2/ 7/27	3.0	96	34	36	3	00		55	0.1	No effect on convulsions
*3/ 7/27	0.5	76	23	73	1	55		45	0.3†	Convulsions abolished
(29)										
2/14/27	0.3	80	60	67	3	30		30	0.1	(No hypoglycemic reaction)
*3/ 9/27	0.3	75	38	88	2	10	1	00	0.3	Convulsions abolished
*3/15/27	0.2	79	39	84	2	00		38	0.2†	Disappearance of convulsions
*4/ 8/27	0.2	72	25	68	2	25		25	0.1†	Convulsions abolished

TABLE 3—*Concluded*

CAT NUMBER AND DATE	INSULIN GIVEN (UNITS PER KILO)	BLOOD SUGAR (1) Initial, 0 minute	BLOOD SUGAR (2) After depression by insulin	BLOOD SUGAR (3) After injection of adrenalin	TIME BETWEEN (1) AND (2) BLOOD SUGARS		TIME BETWEEN (2) AND (3) BLOOD SUGARS		ADRENALIN INJECTED	OBSERVATIONS
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*hrs.*	*min.*	*hrs.*	*min.*	*mgm. per kilo*	
(*30*)										
2/ 2/27	4.0	83	30	67	3	30		55	0.1	Slight weakness disappeared
2/14/27	2.0	80	36	33	3	30	1	35	0.1	No change in condition
(*33*)										
3/23/27	3.5	88	37	39	3	30		25	0.2†	Convulsions abolished
(*34*)										
3/21/27	4.0	77	16		2	00			0.1	Convulsions not affected
(*36*)										
4/11/27	2.5	91	29	41	1	30		15	0.1†	Weakness abolished
(*41*)										
3/18/27	2.0	71	29	42	3	30		14	0.2†	Convulsions abolished

† Intraperitoneal injection; other cases, adrenalin given subcutaneously.

increase was from 27 mgm. to 102 mgm., and the average increase was 53 mgm. Convulsions or hypoglycemic symptoms were abolished in all these cases but one, in which no symptoms were present at the time of administration of adrenalin. In a further experiment (no. 26, January 31) in which convulsions were not affected by a small dose of adrenalin, it is to be noted that the animal had been operated on six days previously and had undergone a number of severe convulsions before adrenalin was given.

Normal animals which had been insulinized, however, showed in eleven cases an average increase of blood sugar following adrenalin injection of only 15 mgm. per cent. Of these, one case which showed an increase of 49 mgm. had suffered only a moderate reaction, another which increased 37 mgm. had shown only slight symptoms, and a third case which showed an increase of 32 mgm. had sustained no hypoglycemic reaction. In all other instances in this series the changes in blood sugar which were evoked by adrenalin were negligibly small. In the normal animals, adrenalin failed to abolish convulsions in three experiments, and in another experiment the hypoglycemic symptoms were not affected.

Examples of the marked differences in glycemic response to adrenalin before and after medulliadrenal inactivation are depicted in figure 2.

The administration of small doses of adrenalin to animals without active medullary tissues, and extremely sensitive to insulin, was observed to provide complete protection against reduction of the blood sugar and the occurrence of hypoglycemic symptoms. A few examples are briefly cited. In cat 23 the adrenal operation was carried out on January 8; on January 19 a severe reaction occurred after giving one-half unit of insulin per kilo,

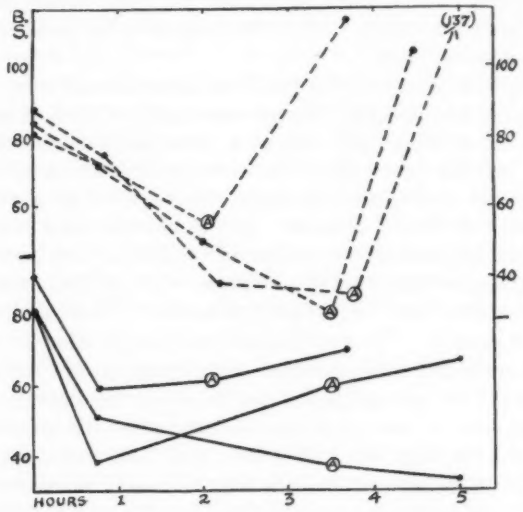

Fig. 2. Typical effects on the blood-sugar level during insulin hypoglycemia of 0.1 mgm. per kilo of adrenalin hydrochloride, given at *A*. Continuous lines, normal controls; dash lines, animals with adrenal medulla inactive. Large increments in the glycemic percentage were observed after adrenalin injection in the medulliadrenal inactivated animals, although less resistance was shown toward insulin (see fig. 1). Insulin given at 0 hour.

while convulsions were produced on January 14, and again on February 18, by 1 unit per kilo. No reaction took place, however, from 1 u.p.k. on February 28 when small amounts of adrenalin were also injected (two doses of 0.2 mgm. p.k. each, given subcutaneously, one at 20 minutes and another 2 hours after insulin). The blood sugar readings in this experiment were 83 mgm. per cent (initial), 81 mgm. (2 hours after insulin), and 145 mgm. (at 3½ hours). A few days later an injection in the same animal of 1 u.p.k. of insulin, without adrenalin, was followed by severe hypoglycemia and convulsive seizures. Another animal, no. 26, which was operated on

(adrenals inactivated) on January 25 and was later found to be extremely and permanently sensitive to insulin, provided further evidence of the ready protection by adrenalin against insulin hypoglycemia. Whereas 1 u.p.k. of insulin alone, on January 31, produced marked hypoglycemia and severe convulsions, on February 28 the same dose of insulin plus small amounts of adrenalin (two doses of 0.2 mgm. p.k. each, given subcutaneously) produced no symptoms and no reduction of the blood sugar. Only a slight reaction and an insignificant and transient fall in the glycemic percentage resulted from 2 units of insulin per kilo (twice the observed convulsive dose), on March 4, when adrenalin also (in dosage as on February 28) was administered.

Liver and muscle glycogen of medulliadrenal inactive animals. The glycogen of the liver and muscular tissues was examined in a series of normal cats, and in others which had undergone medulliadrenal inactivation from one to five months previously. In seven operated animals there were found only slight variations in glycogen content from those observed in a similar number of control animals. In the control cases the percentage determinations for liver glycogen ranged from 2.04 to 4.88 grams and those for muscle glycogen from 0.28 to 1.17 gram, while in the operated animals the variations were from 1.54 to 4.00 grams for the liver and from 0.41 to 1.02 gram for muscle. The examinations were made after the animals had fasted 24 hours and in some instances after severe insulin convulsions had been produced. It was significant that in two of the operated convulsive cases the amounts of liver glycogen observed after the animals had been guillotined were as high as 3.36 grams and 3.96 grams per cent—the latter amount being present, indeed, after a number of severe convulsions had occurred (throughout a period of one hour), and when signs of death were imminent.

SUMMARY

The function of the adrenal medulla in relation to the action of insulin has been considered in cats kept in the laboratory over long periods. After having carried out control observations on the response of normal animals to insulin, medulliadrenal inactivation was performed by the operation of *a*, medullectomy and also by that of *b*, removal of the right adrenal gland and denervation of the left. Both types of operated animals, maintained in vigorous health, exhibited similar and striking changes in susceptibility to insulin. Whereas the hypoglycemic reactions were commonly slight or absent on administering two or (sometimes) four units of insulin per kilo weight before operation, lower blood-sugar values and convulsive seizures were often observed following the injection of one-half or (sometimes) one-fifth of a unit per kilo in the same animals after the adrenal medulla had been rendered non-functional. The suppression of adrenin secretion therefore resulted in a four to tenfold increase in sensitivity to insulin.

The depression of the sympathetic nervous system by the administration of ergotamine tartrate further, but only slightly, accentuated the hypersensitivity of operated animals to insulin.

During hypoglycemic prostration in medulliadrenal inactive animals the convulsive fits were frequent and severe, often interposed by general tonico-clonic contractions, and manifested a tendency to become confluent in character.

The adrenal inactivated animals showed no increase in blood sugar following convulsions and no ability to recover unless assisted by the injection of glucose or adrenalin. In animals with the adrenal glands intact, however, spontaneous recovery from insulin shock frequently occurred.

Small amounts of adrenalin rapidly elevated the blood sugar and promoted recovery from convulsions in medullectomized animals. Correspondingly large increments in the glycemia did not occur on giving adrenalin during convulsions to animals possessing active adrenal tissues.

The increased sensitivity to insulin of medulliadrenal inactive animals persisted without notable change for over four months. Complete protection against lowering of the blood-sugar percentage and insulin shock was found to be readily afforded by the administration at suitable intervals, commencing shortly after the injection of insulin, of small doses of adrenalin.

The liver and muscle of animals that had previously been deprived of the adrenal medulla and were killed during severe insulin hypoglycemia contained normal amounts of glycogen.

The experimental results offer proof that in the absence of the normal adrenin secretion the organism suffers a severe and persistent lowered ability to counteract the effects of minute amounts of insulin. It is further indicated that a highly potent secretion from the adrenal medulla, tending towards depletion of the hepatic glycogen stores in the body while supplying sugar to the blood stream for use by the muscular and other tissues, takes place during insulin hypoglycemia, and that in the absence of this secretion the glycogen depots although well furnished have a much restricted availability to the interdependent parts of the organism.

While life may apparently be normally sustained, as is well known, after suppression of medulliadrenal activity, the experiments demonstrate that under certain critical or emergency conditions a marked incompetence becomes evident. Statements that adrenin secretion is unimportant and without noteworthy influence on bodily activities therefore appear unjustified.

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THE PREPARATION OF A STERILE, ACTIVE EXTRACT FROM THE ANTERIOR LOBE OF THE HYPOPHYSIS

With Some Notes on its Effects[1]

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The origin of the present studies. We may consider that the history of active extracts of the anterior lobe of the hypophysis begins with the work of Evans and Long (1) in 1921. To be sure, previous investigators had, in isolated instances, described effects from feeding anterior lobe substance or the injection of fractional extracts from it, but their results taken all together are overshadowed by Evans' startling discovery, and they need no further mention here.

The precise details of the preparation of Evans' later extracts have not been published. At first, he used merely an aqueous suspension of glands which, under as strict aseptic precautions as was possible had been ground with sand and freed of particles by centrifuging. The resulting fluid, though not sterile, could nevertheless be used intraperitoneally in rats and tadpoles, with the amazing results which are described in Professor Evans' Harvey Lecture in 1925. Briefly, the changes produced consist of a prompt cessation of estrus, and ultimately of a general overgrowth in the adult female rat which may reach two or three times the size of controls. The cessation of estrus appears to be dependent upon a specific change in the ovaries, which accumulate great bunches of corpora. In hypophysectomized tadpoles and young rats, growth is restored.

Other methods of extraction are mentioned in this lecture, including the use of a mild alkali to bring as much of the substance of the gland as possible into solution, with subsequent neutralization. The resulting fluid could be passed through a Berkefeld filter. The active principle could be precipitated by alcohol, and redissolved. Methods of preparation are not described minutely, however, and doubtless much standardization of technique remains to be accomplished, even in the laboratory from which the extract originated.

[1] The expenses of this investigation were defrayed in part by a contribution from the Philip Gray Fund for the Study of Brain Tumors, in part by a grant from the DeLamar Mobile Research Fund.

The great significance of Professor Evans' discovery for physiology and pathology is obvious. A whole new chapter in endocrinology is begun. However, before the study of the anterior lobe hormone can be carried much further in the laboratory, and before its therapeutic usefulness can be tested, two steps seem necessary. The most essential one is, that some definite, quantitative, and if possible rapid test for its presence must be found. Only less important is the standardization of a reliable method of preparing large quantities of a sterile, stable clear solution which is suitable for chemical manipulation and for injection into animals less tolerant than the rat. The two steps depend to some extent upon each other. Unless different samples of extract can be accurately tested and compared, it is difficult to choose between different methods of preparation. And, on the other hand, until a stable, standard solution is available in large quantities, it is difficult to be sure that any given test is specific for it.

Early in 1926, in beginning an attempt to elaborate the discoveries of the California investigators, we found it quite impracticable to prepare daily fresh sterile extracts according to their method. Procuring glands from a distant slaughter house daily, dissecting them under aseptic precautions, carrying out the grinding with sand, alkaline extraction, neutralization and centrifuging, and at the same time maintaining and observing rigidly a colony of rats, is indeed a tedious labor. Moreover, whenever any new chemical procedure was introduced there was the chance of infection and the question whether the particular lot of extract was actually active. By the time a fluid was thoroughly tested, it was usually either contaminated or used up.

During this early period we were able, however, to confirm Evans' chief findings. In female rats estrus stopped promptly, and with few exceptions remained in abeyance during the period of injections. The treated animals gained weight at an average rate of one to three grams a day. One female grew to a weight of 557 grams, where her control never weighed more than 187. It is unnecessary to elaborate the results further, as these experiments were not carried out so thoroughly, so long, nor on such a large scale as those of Evans and his co-workers, and they are mentioned only by way of corroboration.

After a few attempts at modification of the original technique it became obvious that with the facilities available, nothing could be accomplished until an extract could be made in relatively large quantities and preserved over a period of time long enough to test its activity by the production of growth in rats. Since heating destroys the active principle, the simplest method of avoiding infection appeared to be the addition of an antiseptic. But the preservative must be one which is harmless to the animal and one which does not destroy the active principle. Alcohol, phenol, mercurochrome, acriflavine, and hexylresorcinol were tried, but they all

precipitated or destroyed the hormone when in effective concentration—at least, under the conditions necessarily imposed at that time.[2] Finally, a mild but harmless antibacterial substance, compatible with the growth producing principle, was found in sodium benzoate. Extracts containing ½ to 1 per cent of this substance remained in sufficiently good condition to be tolerated intraperitoneally by rats after a week in the ice-box, and for several months at least if frozen. Fluid made and preserved in this way was not sterile, however, and the lot kept on hand for the daily injections might suddenly become so contaminated that all the test rats would become sick from it. A few cubic centimeters injected into a dog never proved fatal, but would always cause the animal to vomit and lose weight. Nor were the solutions sufficiently clear to be used for chemical manipulations. The turbidity, due largely to suspended bacteria, persisted after ordinary filtration. However, the filterability improved when the extracts had stood for a week or more. The fresh suspensions would clog an ordinary filter paper almost immediately.

In spite of the danger of adsorption, the next step seemed to be to find a more efficient means of filtration. A fluid which had stood in the cold room for a week, and then centrifuged and filtered through paper, would pass through a Berkefeld candle, but only slowly and for a short time before the pores were clogged. Fifty cubic centimeters were about as much as a single worker could prepare by this method in a day's time. This slow process enabled us to confirm the activity of a sterile extract on rats, but we were unable to secure enough fluid to use on larger animals.

The problem was finally solved—or partially solved—by the use of the Sieck filter.[3] This is a commercial material, imported from Germany, which consists apparently of a specially prepared layer of cellulose pulp between two pieces of cloth. The "Entkeimungsschichten" are clamped in a metal funnel, and the whole may be sterilized. The fluid is filtered by suction, and comes through perfectly clear, limpid and sterile up to the time the filter clogs. The filtering disc can then be discarded and the process repeated. Whether or not the activity of the extract is decreased by this filtration remains to be determined. But at least some activity remains.

[2] From even our fragmentary knowledge of the chemistry of the hormone, it is clear that none of the commercial preparations at present available can contain it. Dr. Leo Davidoff working in this laboratory investigated the preparation of all the extracts on the market, and found only one ("Antuitrin" of Parke, Davis & Co.) which was not sterilized by heat. "Antuitrin" is distinctly acid, and contains chlorbutanol, which raises a presumption against its possible activity. Through the courtesy of the manufacturers, Doctor Davidoff was able to test the effect of daily injections of this preparation in rats and in one human subject with pituitary deficiency, but obtained no definite acceleration of growth. We have also been unable to demonstrate any specific action from it.

[3] To be obtained from the Empire Laboratory Supply Company, New York.

The method of preparing sterile fluid at present in use may be outlined as follows:

Preparation of sterile anterior pituitary extract. Beef glands are received as fresh as possible from the slaughterhouse; they may, however, be kept frozen for a week or two at least with little damage. The whole gland is split lengthwise and the anterior lobe is shelled out from either side with a broad-bellied scalpel. They are passed through the finest crushing plate (usually used for pulverizing nuts) of a meat-chopper. The finer the grinding, the easier is the rest of the process.

To every 100 grams "mash," 5 grams sodium benzoate and 50 cc. of 1 per cent sodium hydroxide are added, and the whole made up to a liter with tap water. The mixture should stand at least over night, and may stand several days in a cold place. It is perhaps an advantage to have it freeze. Much debris—mostly connective tissue—settles out of it, leaving a pink, turbid solution.

When the orange colored parenchyma cells are well dissolved, and before the adventitious hemoglobin is destroyed, the solution is decanted through glass-wool to dispose of the slimy sediment. The last drops of fluid may be secured from the precipitate by hanging it in a "pudding-bag" of muslin over a vessel for some hours. The solution is then brought to near neutrality—about pH 7.8—by the addition of dilute hydrochloric acid. Phenol red is used as an indicator in samples removed during the process, and a salmon-pink color signifies the end-point. As it is approached the hemoglobin color returns and a fine colloid precipitate comes down, which is often difficult to deal with. Usually it is necessary to leave the neutral fluid on ice for several days or a week before it will filter well. It is better to keep the salt concentration of the mixture as low as possible to favor the settling-out of sediment.

At best, filtration requires much patience. The solution which has stood and cleared in a cold place should be decanted very gently, in order to leave the sediment behind. It is worth while to centrifuge it, either in an ordinary laboratory centrifuge with largest sized cups or in a bench cream separator. The supernatant fluid is filtered through paper and is then ready for the Sieck apparatus. Even after this preparation, a yield of not more than a quarter or half of the rated capacity of the filter may be expected. We have used a one liter filter (14 cm. diameter) and have preserved the fluid in 30 cc. bacteriological test-tubes stoppered with paraffined cotton, of course under sterile conditions.

If a "batch" of fluid becomes infected, as may easily happen, it may readily and quickly be refiltered. Meanwhile it is best kept frozen. The presence of a few saprophytes seems to make little difference.

The optimum dose remains to be determined. We have employed 1 to 2 cc. per kilo of animal daily in dogs but up to 4 cc. may be given to a rat. It is ordinarily used intraperitoneally, but is tolerated subcutaneously.

It is not to be supposed that this is the only method of securing a satisfactory fluid, nor necessarily the best way. It appears to be the simplest method at present available. The ground glands have a distinctly acid reaction, and it is necessary to add alkali in order to get a fluid which is not irritating. A better yield seems to be obtained if a slight excess is used. The fluid is not well tolerated if its reaction falls outside of the limits of change of phenol red. Moreover, extracts more acid than pH 6 appear to have little or no activity, even if later neutralized. The active principle

may be precipitated and redissolved in various ways which dispose of some of the contaminating protein, but so far these methods have not produced a more potent or better tolerated product. It is not even certain if the solution prepared according to the outline given is saturated in respect to the active principle, or if any remains behind in the residue, but this question must await the advent of a quantitative test. So far attempts to concentrate it by evaporation have been failures. The optimum dose also remains to be determined.

Uses and properties of the fluid prepared. Owing to the courtesy of Dr. E. M. Houghton, Director of the Biological Laboratories of Messrs. Parke, Davis & Co., we were furnished with an ample supply of glands as fresh as their transportation would permit. For special uses glands from a local abattoir were also employed. We have thus been enabled to prepare and sterilize a liter or more of extract a week.

Each lot of extract is tested for its ability to cause growth in adult female rats. In general, we have found it best to keep a small colony of rats, using each one for several lots of fluid in succession, so that its individual response may be well known. Furthermore, rats which have been receiving injections of active fluid are more sensitive test-objects than are fresh animals, as they lose weight promptly when changed to an inactive one. Occasional extracts have appeared inactive, whether on account of some difference in the glands used or on account of some unrealized variation in the method of preparation is impossible to say. The activity does not seem to be affected by the time of year. During the last few months, as familiarity with the method of preparation has been gained, all of the extracts made according to the formula given have displayed activity.

With this extract, acceleration of growth has been produced in rats and (so far as we know, for the first time) in dogs. The injections must be given to dogs for many months before any effect is observed, and there is always the chance that the animal or its control will succumb to distemper or mange. One thoroughbred bull-dog has received injections daily for eight months, however, and has gained progressively in weight over its littermate sister. The treated dog at present weighs 26.7 kgm., the control 18.2. There have been certain acromegaloid changes in the injected animal which will be made the subject of a later report. It may suffice here to reproduce out-line drawings of the x-ray films of the skulls of the injected animal and its control.

The extract has been administered to rabbits also, but no increase of growth was obtained, possibly because of intercurrent disease.

In hypophysectomized dogs and rats, restoration of growth has been produced in preliminary experiments. The growth of shaven hair and the healing of wounds is much delayed after hypophysectomy, but these processes may be caused to reappear by administration of the hormone.

So far, we have been unable to demonstrate any effect on the metabolism of normal animals (rats, rabbits, guinea pigs, opossum, frogs, crocodiles). The metabolism of hypophysectomized dogs and rats, and the effect of injection upon it is under investigation. Much to our disappointment, we were unable to confirm Winton and Hogben's (2) statement that hypophysectomy lowers the metabolic rate of the frog, or to demonstrate any action of anterior lobe extract upon them after hypophysectomy.

The extract prepared as described will apparently arrest estrus in the rat as effectively as Evans' original emulsion. But it must be admitted that we have paid relatively little attention to this effect of injections, as the rats in our colony have not had cycles with perfect regularity; moreover, estrus can be arrested by the intraperitoneal injection of non-specific irritating substances. The extract will also bring on estrus in the immature rat, as had already been noted by Teel (3). Smith (4), and Zondek and Ascheim (5) have independently observed the same effect in the infantile mouse after gland transplantation, and the latter have proposed it as a test for the hormone. But Smith (6) finds that the injection of Evans' extract does not restore the degenerated sexual system in hypophysectomized animals. So far, we are unable to add anything to these authors' observations.

The sterile tested extract has also been used in one case of pituitary insufficiency in a human being, through the courtesy of Doctor Cushing, under whose care the patient was:

> The patient, a pituitary dwarf five years of age, was an extreme example of hypophysial insufficiency due to a congenital craniopharyngeal pouch cyst. When three years of age, the cystic portion of the lesion had been removed at operation with subsequent freedom from pressure symptoms but, after an interval, with progression of her insufficiency syndrome. What was obviously needed was substitution therapy and in the hope of supplying this she was given seven injections (95 cc. in all) of sterile anterior lobe extract in the course of four weeks. The injections caused temporary diarrhoea and vomiting and though each of them was followed by unquestioned improvement in the child's general condition shown by alertness, relief of headaches, and increase of appetite, she failed to gain in weight or height.
>
> A day or two after the last injection, her general condition grew much worse—so bad that further glandular treatment was out of the question. A week later, she suddenly went into coma one morning, vomited, became cyanotic, developed a shallow, rapid respiration, and died within eight hours. Autopsy showed the persistence of a small cyst about the size of a plum overlying the sella, and the usual visceral changes of the disease. There was no sign of peritonitis or injury to the abdominal contents.

This one case, in spite of its fatal outcome, goes to show that this or similar extracts may be used in the human being without grave danger, and with perhaps some benefit. It shows also that its administration is accompanied by certain difficulties and discomforts which should make us cautious about its use. Further clinical studies are now in progress.

Specific tests for the anterior lobe hormone, and its standardization. This problem still remains without a satisfactory solution. We have depended entirely upon the production of growth in normal adult female rats, but the method is slow (two weeks at least) and is not quantitative. The cessation of estrus is not dependable, for the reasons already given. It remains to be seen whether the production of estrus in immature animals is

Fig. 1. Superimposed tracings of radiographs of the skull of a ten months old thoroughbred bulldog which has received daily injections of 10 to 30 cc. anterior lobe extract (in dotted line); and that of her untreated litter-mate sister (in thin continuous line).

due to the same hormone as that which produces growth, and if the Zondek-Ascheim test is as satisfactory for aqueous extracts as for experiments with gland transplantation. The same question may be raised in regard to Teel's placentoma reaction (3). The effect, if there is any, of the anterior lobe hormone on the basal metabolism of normal animals is so slight, that it is out of the question as a test. So far, blood chemistry studies have

yielded no definite results, although by analogy with cases of acromegaly some change might be expected in the blood sugar.

The most promising test appears to be the production of growth in hypophysectomized rats, which has been used by Smith (7) in estimating the efficacy of gland feeding. The effect on the healing of wounds and the growth of hair in these animals may also prove a specific and satisfactory test. Or the acceleration of metabolism in them might be developed as a more rapid and perhaps quantitative method. It is along these paths that our chief efforts are now being directed.

SUMMARY

1. A method is described for the preparation of a sterile active extract of the anterior lobe of the hypophysis.

2. This extract has been used in rats and dogs with the production of changes which appear to be specific.

3. It serves to repair some and perhaps all of the disabilities produced by hypophysectomy in these two animals.

4. The extract has been used in one human case of pituitary insufficiency from a craniopharyngeal pouch cyst, with apparent temporary benefit.

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BLOOD AND CEREBRO-SPINAL FLUID CHANGES AFTER INTRAVENOUS INJECTION OF HYPERTONIC SOLUTIONS[1]

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The use of hypertonic solutions for the reduction of increased intracranial pressure through the decrease in the brain volume is now a matter of routine clinical application. It has been assumed that the decrease in brain volume is caused by a withdrawal of water from its substance with liberation into the blood stream. There is experimental evidence that other organs too, notably the muscles, give up part of their water content at the same time. It is assumed, therefore, that the water content of the blood, and hence its volume, is thereby increased. Experimental evidence pointing to this is fairly abundant, but apparently only one investigator (Lazarus-Barlow, 1) has paid close attention to the extent and rapidity of this change.

The present study was undertaken: *1*, to determine the rapidity and extent of the changes in the water content of the blood; *2*, to determine whether there was any loss of water from the cerebro-spinal fluid; and *3*, to correlate them, if possible, with the changes in cerebro-spinal fluid pressure.

HISTORICAL. *Cerebro-spinal fluid changes.* Weed and McKibben's original work (2) upon the effect on the cerebro-spinal fluid of intravenous injection of hypertonic solutions has been confirmed by numerous observers and amplified chiefly in its clinical aspects. No essential variations from their original conclusions are found in the literature. Foley and Putman (3) demonstrated the efficacy of sodium chloride by mouth as a means of reducing the intracranial tension. They also concluded from indirect evidence that hypertonic sodium chloride intravenously, as well as sodium chloride by mouth, produces a change in the ratio of absorption to secretion of the cerebro-spinal fluid, as well as in the brain bulk.

Ebaugh and Stevenson (4) and T. Fay (5) demonstrated a secondary increase of cerebro-spinal fluid pressure which they called a wave of edema following the ingestion of sodium chloride by mouth. This edema they

[1] This investigation has been assisted by a grant from the Ella Sachs Plotz foundation.

concluded to be due to the storing up of sodium chloride in the brain tissues, which after a certain concentration is reached becomes of a greater tonicity than the blood, and hence directs fluids again into the brain tissue. They found that with hypertonic glucose, this secondary wave of edema did not appear. They explained this observation by the fact that the diffusion time of glucose is three times that of sodium chloride, and its osmotic pressure is proportionately less. They also conducted careful urinalysis after the intravenous injection of 200 cc. of 30 per cent glucose and found that no glycosuria resulted.

Blood changes. In previous experiments to determine the changes in blood volume and water content that occur following the intravenous administration of hypertonic slutions, the nature and degree of these have been estimated by one or more of five methods: 1, total blood volume; 2, hemoglobin estimations; 3, serum protein content determined by the refractometer; 4, cell volume percentage determinations (hematocrit); and 5, specific gravity determinations on whole blood and serum. Underhill and Segal (6), using the hemoglobin method, found a decrease in hemoglobin content very shortly after the injection of either glucose or NaCl solutions; this persisted for thirty or forty minutes before it returned to normal. No attempt was made, however, to estimate quantitatively the amount of water added from the tissues; nor does this seem practicable when one considers that hemoglobin estimations are themselves notoriously unreliable.

H. P. Smith (8) using blood volume methods, in which he claimed to have eliminated the error caused by retention in the blood of dye from the first injection, noted a definite and considerable increase in blood volume, above that to be expected from the mere addition of the injected fluid. In none of these experiments quoted, however, has any attempt been made to show quantitatively the net change in blood volume after due allowance for the amount of fluid injected.

Lazarus-Barlow (1) showed that in dogs, following the intravenous administration of hypertonic sodium chloride, dextrose and urea, there was an immediate drop in the specific gravity of whole blood and serum, (using Roy's method), within one minute after the injection was completed. This was more pronounced following saline than dextrose. He used 18 per cent saline and 55.38 per cent dextrose (Sp. Gr. 1.185). The concentration of the blood gradually returned to normal, and in some cases became higher than the previous normal level; but following repeated injections, the concentration became less and less, and remained at a much lower level than before. The changes were parallel in whole blood and in serum. In the one experiment reported by him in detail, using NaCl, the blood volume was increased by 14.5 per cent, according to our calculations (see discussion). He also found that the specific gravity of volun-

tary muscle increased, indicating water loss. His experiments, however, were all carried out under a condition that in itself produces very marked changes in blood concentration,—namely, under ether-chloroform anesthesia (Barbour and Bourne, 9).

Weed and McKibbon (2), Cushing and Foley (10), and others have shown that there is loss of water from the brain following the intravenous administration of hypertonic solutions. Baer (11) demonstrated a similar loss from muscle tissue, when the hypertonic solution was given by mouth.

Starling (12) says that following hypertonic injections, a shrinkage of the limbs and a swelling of the abdominal viscera can be observed, indicating that muscle in particular loses water; that all of the tissues in the body except the abdominal organs, shrink in volume. He also states that the fluid that enters the blood from the tissues is really physiological salt solution, and that the effect is the same as if there were injected with the hypertonic solution, a larger quantity of physiological salt solution.

METHOD. Our observations were carried out on eight humans and six dogs. The humans had cranial injuries, usually fractured skulls, and most of them had increased intracranial pressure. No anesthetic was used in the human group; some of the patients were semi-comatose, others perfectly conscious. The patients were placed on their left side and remained there during the whole of the period of observation. Some of them remained quiet throughout; in others (noted in connection with data below), coughing, vomiting, or outbursts of delirium affected the spinal fluid pressure readings considerably. A lumbar puncture needle was introduced into the lumbar space, and when fluid appeared, a manometer was attached directly, on a threeway stop-cock, with the loss of no more than a drop or two of fluid, at the most. Readings of the height of the cerebro-spinal fluid column were then made at frequent intervals. Meanwhile, in all but one experiment (ear samples), blood was obtained from one of the arm veins; no tourniquet was used, the needle being inserted against the blood flow, and very transient pressure being applied on the vein above the needle until the latter was inside the vein.

In two of the experiments, a portion of the blood was also added to sodium citrate crystals, to be used in determining the cell volume percentage (hematocrit).

When the cerebro-spinal fluid pressure had reached a more or less constant level, and after preliminary blood samples had been obtained, the dextrose or saline injections were started. These were given, usually with a 20 cc. syringe, into one of the arm veins. The saline used was usually 90 cc. of a solution of which 100 cc. contained 15 grams NaCl crystals. The dextrose was, in the earlier cases, 250 cc. of a solution of which 100 cc. contained 25 grams dextrose; in the later cases, 125 cc. of a solution of which 100 cc. contained 50 grams dextrose.

In all the experiments cerebro-spinal fluid pressure readings were continued during and following the injections. In some of the cases, blood samples were taken, exactly as before the injection, 1 minute, 15 minutes and 30 minutes after the injection; in other cases, they were taken also while the injection was proceeding, the other arm being used except in the one case where puncture of the ear was used throughout. Observations on cerebro-spinal fluid pressure and on blood were continued for 30 to 90 minutes following completion of the injection.

In the dog experiments, it was found necessary to give either morphine or amytal, or both to keep the animal quiet. Cisterna puncture was used in all instances. The blood, in all cases, was obtained by cutting an ear vein, which could be made to bleed freely throughout the experiment by rubbing briskly with cotton or a sponge. The specific gravity of the whole blood was determined at once. After the cerebro-spinal fluid pressure and the specific gravity of the blood had reached a uniform level, the injection was begun. Varying quantities of dextrose were given prepared in the same way as for the human cases. It was administered by gravity, sometimes augmented by pressure, from a graduated flask or bottle, into the right ventricle of the heart. The time of injection varied from seven to ten minutes. In all of the experiments, repeated observations on blood concentration and cerebro-spinal fluid pressure were made during, as well as following the injection.

On two dogs and one human (a later experiment) the specific gravity of the cerebro-spinal fluid was measured before the injection was given, using in the dogs a minimal amount of fluid, so that no more than a drop at the most was lost. As the cerebro-spinal fluid pressure reached its lowest level, a second determination was made, the fluid being obtained in this way:—jugular pressure was applied, and the column of cerebro-spinal fluid was raised as high in the manometer as it would go. This washed out the needle, filling it with fluid fresh from the subdural space. The stop-cock was then turned until a drop or two of fluid flowed from the needle.

The specific gravity determinations on whole blood, serum and cerebro-spinal fluid were made by the method of Barbour and Hamilton (13). The probable error by this method is not greater than ± 0.0001, or one point in the 4th decimal place. The cell volume percentage (hematocrit) was determined by centrifugalization in standard graduated centrifuge tubes at the same speed for exactly 30 minutes. In one case, total solids were determined as a check on specific gravity of serum, by evaporating to dryness at 100° to 110° for 24 hours. This determination was made on serum before injection, and a sample one minute after injection.

RESULTS. *Cerebro-spinal fluid pressure.* In most cases both in animals and humans, the pressure rose temporarily, while the injection was in

progress, falling below its previous normal level, later. The extent of the rise depended partly on the disturbance to the animal or subject occasioned by the procedure. In many cases, however, there was no disturbance, and the rise seemed to be entirely independent of this. The extent of the fall varied considerably. In the human cases, it was slightly greater with saline than with dextrose, but in one dog, it fell to zero following 50 per cent dextrose. Altogether, the fall in pressure was very much greater in the humans than in the animals; the volume of fluid injected seemed to have little influence in determining its extent. Table 1 shows

TABLE 1

Changes in cerebro-spinal fluid pressure following the intravenous administration of hypertonic solutions of dextrose and NaCl

	EXPERIMENT	CEREBRO-SPINAL FLUID PRESSURE			SUBSTANCE INJECTED	CONCENTRATION	AMOUNT	CONDITION OF SUBJECT
		Low before injection	Low after injection	Fall in pressure				
						per cent	*cc.*	
Humans	1	180	100	80	Dextrose	25	250	Quiet throughout
	2	100	40	60	NaCl	15	90	Vomited during 2 minutes
	3	220	55	165	NaCl	15	90	Quiet throughout
	4	190	120	70	NaCl	15	90	Quiet throughout
	5	110	60	50	Dextrose	50	125	Quiet though talking
	6	130	80	50	NaCl	15	70	Quiet throughout
	7	130	65	65	Dextrose	50	150	Irrational, moved
	8	70	40	30	Dextrose	50	150	Restless
Dogs	9	100	50	50	Dextrose	50	25	Quiet—morphine
	10	70	45	25	Dextrose	50	60	Quiet—morphine and amytol
	11	110	100	10	Dextrose	25	100	Quiet—morphine and amytol
	12	130	110	30	Dextrose	50	100	Quiet—morphine and amytol
	13	80	0	80	Dextrose	50	150	Quiet—morphine and amytol
	14	100	76	24	Dextrose	50	80	Quiet—morphine and amytol

the lowest pressure before injection, and the lowest after. In column 4 is shown the extent of the fall. In experiment 3, it will be noticed that a fall of 165 mm. took place; it is felt that in this particular experiment these figures are not conclusive because the initial low level was not established, since the pressure began to fall rapidly even before the fluid was injected. As a whole, when the pressure was very high to start with, it dropped much more than when it was low, irrespective of whether glucose or saline was the fluid injected. Where the pressure was originally low, there seemed to be little difference in the degree of the fall, whether glucose or saline was

used. We did not in all cases determine the duration of the lowered pressure. In one human, it returned to normal 35 minutes after the injection was completed. In another, it was still low 90 minutes afterwards.

Blood concentration. In every case there was a marked and immediate fall in specific gravity of both whole blood and serum. In the earlier experiments, where blood was taken after the injection was completed, the first sample showed the greatest dilution, with steady concentration

TABLE 2

Changes in specific gravity of blood, serum and cerebro-spinal fluid, following the intravenous administration of hypertonic solutions of dextrose and NaCl

EXPERIMENT	WEIGHT	SPECIFIC GRAVITY				HEMATOCRIT		SERUM SOLIDS		CEREBRAL-SPINAL FLUID, SPECIFIC GRAVITY		SUBSTANCE INJECTED
		Blood		Serum		Before	After	Before	After	Before	After	
		Before	After	Before	After							
						per cent	*per cent*	*per cent*	*per cent*			*per cent*
1		1.0581	1.0547			47.9	41.5					Dextrose 25
2		1.0604	1.0561	1.0255	1.0229	47.3	40.1					NaCl 15
3		1.0583	1.0554	1.0277	1.0249							NaCl 15
4				1.0288	1.0260							NaCl 15
5				1.0274	1.0254							Dextrose 50
6				1.0257	1.0229			9.18	8.05			NaCl 15
7		1.0565	1.0488	1.0263	1.0231							Dextrose 50
8		1.0655	1.0610									Dextrose 50
9												
10	11 kgm.	1.0489	1.0442									Dextrose 50
11	18 kgm.	1.0553	1.0486									Dextrose 25
12	20 kgm.	1.0554	1.0498									Dextrose 25
13	15 kgm.	1.0646	1.0484							1.0089	1.0095	Dextrose 50
14	18 kgm.	1.0556	1.0486							1.0080	1.0089	Dextrose 50

The figures under specific gravity represent the lowest reading before the injection was started, and the lowest after; this latter, is the first sample taken after the injection is complete, or in one or two cases, the last one taken while the injection was in progress. The cell volume percentage (hematocrit) and the single serum solids determinations correspond closely to the specific gravity readings and serve as a check on them.

in the succeeding ones. The initial dilution was so marked that in three human and all the animal cases, done later, the blood was taken during the progress of the injection. These showed in every case a dilution beginning immediately after the injection had started. The changes were consistent in concentration of whole blood and serum, serum solids (1 case) and hematocrit. A summary of the changes is given in table 2.

No attempt was made to follow the duration of the period of dilution

of the blood. In none of the human cases did the concentration of the blood or serum return to normal within one hour (the longest period of observation). In one of the animals, after a minimum specific gravity of 1.0486, from an original level of 1.0556, the blood concentrated to 1.0577, 20 minutes after the injection was complete. As the animal was quiet throughout, restlessness cannot account for this. Typical curves of blood

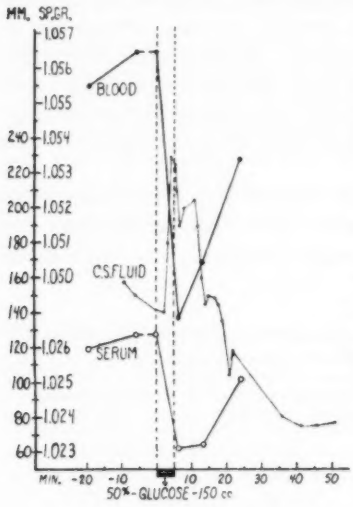

Fig. 1

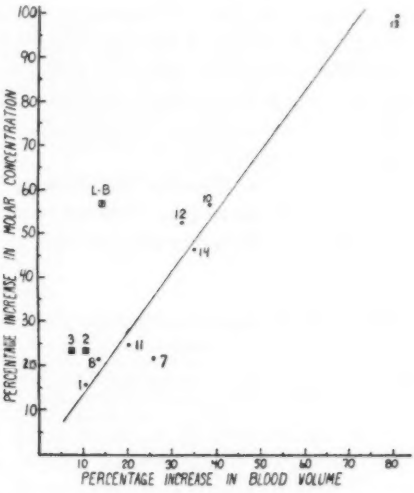

Fig. 2

Fig. 1. Man, fractured skull. Curve showing changes in blood specific gravity (●——●); serum specific gravity (○——○) and cerebro-spinal fluid pressure (●——●), following the intravenous administration of 150 cc. of 50 per cent dextrose solution. The period of injection is represented by the solid rectangle and the broken lines extending vertically from each end.

Fig. 2. The relationship between the percentage increase in blood volume (abscissae) and the percentage increase in molar concentration of the blood with reference to the substance injected (ordinates), following the intravenous injection of solutions of dextrose (●) and of saline (⊡). The numbers refer to the numbers of experiments as given in the tables.

changes are shown in figure 1. None of the other curves varied from these in general shape, though there were fairly wide variations in the degree of the changes.

Cerebro-spinal fluid concentration. In the two experiments on dogs, in which this was determined, there was a definite concentration of the cerebro-spinal fluid, from 1.0089 to 1.0095 in one case, and from 1.0080 to 1.0089 in the other. Neither of these samples showed any trace of

blood. Since the accuracy of the specific gravity method has been shown to be to one point in the fourth decimal place, and since these changes are 6 and 9 points respectively in the fourth place, they are considered to be of significance. One determination was made on a human, which likewise showed a well-marked concentration, but this result was not considered entirely satisfactory, because the fluid was blood-tinged.

DISCUSSION. *Blood changes.* The method used in these experiments of determining the concentration of the blood is, we feel, far superior to the older methods, in both accuracy and speed. It brings out the extreme rapidity with which water is withdrawn from the tissues into the blood stream, while hypertonic solutions are being administered intravenously. This mechanism seems to have achieved its limit almost immediately the injection is complete.

To determine whether the changes that take place in the concentration of the blood are simply additive or whether some other factor enters, we performed in vitro experiments on the density of a mixture of whole blood

TABLE 3

"In vitro" experiments to determine effect on specific gravity of blood of the addition to it of dextrose solution in the proportions used "in vivo"

SAMPLE OF WHOLE BLOOD, SPECIFIC GRAVITY 1.0484	DEXTROSE, 50 PER CENT SPECIFIC GRAVITY 1.1703	OBSERVED SPECIFIC GRAVITY OF MIXTURE	CALCULATED SPECIFIC GRAVITY
cc.	*cc.*		
70	3	1.0530	1.0536
90	3	1.0523	1.0523
110	3	1.0512	1.0516

and dextrose solution. Assuming the total blood volume of an average man to be 5500 cc.: to 110 cc. of whole blood ($\frac{1}{50}$ of calculated blood volume) was added 3 cc. ($\frac{1}{50}$ of volume injected) of 50 per cent dextrose solution. The resultant specific gravity was then determined. As a further check, similar determinations were made using 70 and 90 cc. of whole blood. These correspond respectively to 3500 and 4500 cc. of total blood volume, and 150 cc. of dextrose solution. The results appear in table 3.

It is apparent, therefore, that the addition of dextrose to blood does not alter the density of any of the constituents of the resulting mixture. Hence it is possible to estimate the approximate amount of fluid entering the blood from the tissues provided we know its specific gravity. Starling regards it as the equivalent of physiological salt solution. On this basis, the total amount of fluid withdrawn from the tissues, following the injection of dextrose or saline will be

$$\frac{V\ (v - v_1)}{v_1 - x}$$

Where V = volume of blood before the injection plus volume of dextrose or saline injected.

v = specific gravity corresponding to V.

v_1 = final specific gravity of blood-dextrose (or saline)-tissue fluid mixture.

x = specific gravity of the fluid (physiological salt solution) withdrawn from the tissues.

Assuming original blood volume to be 5500 cc. ($\frac{1}{13}$ of body weight) (12) and applying the equation to experiment 7, the volume of blood plus dextrose will be 5650 and its density (as figured in above "in vitro" tests) would be 1.0595. Substituting these figures in the above equation, and assuming the specific gravity of the physiological salt solution to be 1.0068 (determination of Ringer's solution), the volume of fluid withdrawn from the tissues will be

$$\frac{5650\ (1.0595 - 1.0488)}{1.0488 - 1.0068} \text{ or } 1439 \text{ cc.}$$

If the total blood volume is less than 5500 cc., the result will be less; and if more it will be greater.

The specific gravity of our 15 per cent sodium chloride solution was found to be 1.1018; and of our 25 per cent dextrose solution, 1.0868.

Table 4 shows the results of our experiments in all cases where determinations were made of specific gravity of the whole blood. The figures are given in terms of tissue fluid absorbed from the tissues into the blood expressed as per cent of the original blood volume, which is calculated on the basis that blood constitutes $\frac{1}{13}$ of the body weight. In the humans, it is assumed that 5500 cc. represents an average figure, since all of the patients weighed in the neighborhood of 70 kgm. The calculated molar concentration (in terms of the substance injected) of the mixture of blood and injected solution is given in column 10. Column 11 shows the percentage increase over normal blood. For purposes of comparison, Lazarus-Barlow's results are included. The data from other reported experiments were not sufficiently complete to be used in the same way.

If the amount of fluid withdrawn from the tissues bears a direct relation to the increase in molar concentration of the solid in the blood stream, then these figures, plotted against each other, should lie on a straight line. Such a relation is shown graphically in figure 2, where the ordinates represent percentage increase in molar concentration, and the abscissae percentage increase in blood volume, using the figures in table 4. It will readily be seen that a straight line can be drawn near which practically all of the points lie. This indicates, therefore, that the amount of fluid withdrawn from the tissues is in direct proportion to the increase in molar concen-

tration of the dextrose in the blood stream. The same relationship does not appear to hold true with NaCl, although our experiments with this were too few to draw any definite conclusions; however it appears that a greater increase in molar concentration for NaCl in comparison with dextrose is necessary to produce a corresponding increase in blood volume.

Cerebro-spinal fluid changes. The experiments demonstrate also that water is withdrawn from the cerebro-spinal fluid directly. When one considers the accepted theories upon the formation and absorption of the

TABLE 4

Ratio of calculated increase in blood volume to calculated increase in molar concentration of blood following intravenous administration of hypertonic solutions of dextrose and NaCl

NUMBER OF EXPERIMENT	BODY WEIGHT	BLOOD VOLUME*	CALCULATED TISSUE FLUID ABSORBED	INCREASE IN BLOOD VOLUME	INTRAVENOUS INJECTION				BLOOD + INJECTED SOLUTION	
					Substance	Volume	Concentration		Molar concentration‡	Increase in molar concentration over normal
	kgm.	*cc.*	*cc.*	*per cent*		*cc.*	*per cent*	*mols*	*mol*	*per cent*
3		5500	403	7.3	NaCl	90	15	2.565	0.203	23.8
2		5500	336	10.1	NaCl	90	15	2.656	0.203	23.8
1		5500	576	10.5	Dextrose	250	25	1.39	0.351	15.5
8		5500	740	13.5	Dextrose	150	50	2.78	0.370	21.7
†L-B	11	792	115	14.5	NaCl	26	18	3.08	0.257	56.7
11	18	1312	297	20.4	Dextrose	100	25	1.39	0.380	25.0
7		5500	1439	26.2	Dextrose	150	50	2.78	0.370	21.7
12	20	1459	475	32.5	Dextrose	100	50	2.78	0.463	52.3
14	18	1312	462	35.2	Dextrose	80	50	2.78	0.445	46.4
10	11	806	312	38.7	Dextrose	60	50	2.78	0.476	56.3
13	15	1083	875	80.7	Dextrose	150	50	2.78	0.605	99.0

* Calculated on the basis that blood constitutes $\frac{1}{13}$ body weight.

† Present authors' calculation.

‡ Normal molar concentration of blood in terms of dextrose is 0.3038 mol.
Normal molar concentration of blood in terms of NaCl is 0.1639 mol.

cerebro-spinal fluid, it is not surprising that the fluid should lose its water in the same way that all soft tissues of the body apparently do. This does indicate, however, that the absorptive process is a selective one since water is withdrawn much more rapidly than the solid content of the fluid.

The changes in the cerebro-spinal fluid pressure are not striking in all instances. They bear out, however, the results of other investigators, and so require no further discussion. However, since there seems to be slight appreciable difference in the efficacy of dextrose versus saline in reducing the pressure; and since clinically dextrose is productive of no

apparent ill effects on the patient, while saline may be, we feel that for practical purposes, dextrose is preferable to saline for use in lowering intracranial pressure.

SUMMARY

1. Following the intravenous administration of hypertonic solutions, there is a temporary rise in the cerebro-spinal fluid pressure, which is followed by a consistent fall, varying in degree with the amount and the hypertonicity of the solutions injected.

2. There is a marked and immediate fall in the specific gravity of both whole blood and serum, following the intravenous administration of hypertonic solutions.

3. An estimate is made of the volume of fluid which enters the blood from the tissues. We calculate this volume to vary in man from 300 to 1000 cc. (from 7 per cent to 19 per cent of blood volume), and in dogs from 100 to 900 cc. (from 14 per cent to 80 per cent of blood volume).

4. Close correlation is shown (in the case of dextrose) between the percentage increase in blood volume and the percentage increase in molar concentration of the blood in terms of the substance injected.

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OBSERVATIONS ON PARATHYROIDECTOMIZED DOGS, WITH PARTICULAR ATTENTION TO THE REGIONAL INCIDENCE OF TETANY, AND TO THE BLOOD MINERAL CHANGES IN THIS CONDITION[1]

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There have been made suggestions of a regional relation of disturbances of parathyroid function, although these have never, so far as we are aware, appeared in the literature. It is the purpose of this paper to report the results of an investigation of this possibility, together with data on the relation of blood calcium and phosphorus to the incidence of tetany and other symptoms following parathyroidectomy in dogs.

Thirty-five dogs have been subjected to complete thyro-parathyroidectomy and studied during the period of survival. Animals were under observation for varying periods before operation. During this time, as well as after operation, they received only distilled water, together with a diet of cooked meat and bread scraps, the latter making up approximately two-thirds of the total diet by weight. Variations of this diet were made only as indicated hereinafter.

Observed symptomatology varied greatly in different animals and in the same animal at different times, both in kind and in degree of severity. Neither epiphora nor rhinitis were noted in any case, salivation very rarely. Exophthalmia and pupillary dilatation commonly occurred, particularly before and during an attack of tetany. Another symptom frequently noted, which does not seem to have been recorded by the earlier investigators, was a tendency to project the nictitating membrane across the eyeball. So far as could be determined, this was first described by Hastings and Murray (1921) in dogs and by Nicholas and Swingle (1924) in cats.

Rectal temperature was not greatly elevated except during severe and prolonged tetany, and then inconstantly. Dog 24 died in severe tetany with a rectal temperature of 44°C. In this connection, attention is called to the report of Ogawa (1925) that parathyroidectomized rats lost temperature regulating power.

[1] The expenses of this investigation were borne, in part, by a grant from the Research Committee of the American Association for the Advancement of Science.

Laryngospasm was never observed in adult dogs but was a most common manifestation in puppies. Neither diarrhea nor vomiting occurred frequently. Cardiac and respiratory symptoms were quite variable. A survey of available literature on the less constant and obvious symptoms prompts us to agree with Hastings and Murray (1921) that not enough attention has been given to correlating these factors with the occurrence of the more commonly observed symptoms and sequelae of this operation.

It is particularly significant that no. 36, a very old dog, showed very little symptomatology at any time except uncomplicated tetanic muscular tremors which were very severe at times. While determinations of blood calcium and phosphorus indicated a profound degree of disturbance, the animal never seemed to be particularly distressed by the most severe tetany. This was a very intelligent dog, easily trained to mount scales or operating table at command, both of which performances were as readily attempted during severe attacks as at other times. Even in an attack so severe as to render it difficult to stand, he readily manifested an inclination to play. The significance of these observations will be discussed in another connection. This animal continued active and in good flesh up to the 97th day after operation when he was found dead with no evidence of an attack of tetany.

TABLE 1

Death known to have been associated immediately with an attack of tetany	8
Cause of death undetermined	6
Death from distemper	16
Intentionally killed	2
Accidentally lost	1
Death from overdose of parathormone	1
Alive October 21, 1927, 189 days after operation	1

Table 1 shows the condensed history of the entire series so far as could be determined. Of the series of 35 dogs, 12 were recently weaned puppies of 4 separate litters, numbering 1, 2, 3 and 6 members respectively. Other members of these litters were used as controls. Except for no. 36, all others were young adults, in good condition at the time of operation, so far as could be determined.

Out of more than 50 unoperated dogs confined to the same quarters for periods of a week or more, only 3 ever developed distemper, while 18, or 54 per cent, of these operated contracted this condition and it is probable that all but two died of this cause since autopsy showed the lungs to be invariably congested to a marked degree, and in some instances completely solidified.

These observations are in accord with that first made by Vassale and Generali (1900), later confirmed by Carlson (1912a) and since repeatedly confirmed by others, that parathyroidectomized animals are less resistant to infection.

Two dogs were killed, no. 1 for autopsy, no. 8 because of incurable mange. Nos. 1, 5, 6 and 7 were never observed in tetany and it is believed they never suffered an attack. The three latter were never treated and did not develop tetany but all showed symptoms of distemper on the second or third day after operation. Four other dogs were never treated in any way and all 4 died in 3 to 13 days. No. 2 was never observed in tetany but died on the 13th day in coma of several hours duration, the cause of which was never satisfactorily determined but which was not due to distemper. With the exception of no. 4, which was accidentally lost 3 months after operation and which had recovered from a tetanic period of 3 weeks duration without treatment, all treated animals displayed a frequency and severity of attacks that renders it a fair assumption that they would all have died much earlier except for intervention. Even with treatment, all those known to have died during or after an attack were probably preserved only a few days, at most. Tetany appeared in from 2 to 4 days after operation, except in case of no. 8 which showed no evidence of an attack until the 10th day.

Post-mortem examination for accessory parathyroid tissue was made in every case except no. 4 which was lost, and no. 43, still alive, but none was found except in no. 1.

No. 1, a young adult female, 7 kilos, operated November 20, 1925. Did not develop tetany but on the 3rd day showed a threshold of excitability to galvanic stimulation of 0.5 ma., CCC. From this time on there was progressive recovery until at the end of a week she was normal. Continued to thrive and showed no further disturbance except slight roughening of the coat and dryness of the hair after about 3 months, which persisted during the period of observation. February 25, 1927, plasma calcium concentration was 11.33 mgm., inorganic phosphorus 4.97 mgm. March 31st, Ca, 11.9; P, 6.93. On this date the animal was killed by bleeding and the neck region examined minutely. Old linen ligatures permitted accurate localization of the original site of the thyroid lobes. No thyroid tissue was found. On the left side, posterior to, and approximating the middle of that lobe, but deep in the tracheo-esophageal groove and well removed from the thyroid capsule was found a globular body 2 mm. in diameter, which histological examination showed to be parathyroid tissue, normal in every way and showing no evidence of hypertrophy or hyperplasia.

The immediate cause of death in uncomplicated cases seems to vary. Nicholas and Swingle (1925) suggested that when death does not occur as an immediate accompaniment of tetany, it is the result of depression. In two animals of this series, the immediate cause was probably hyperpyrexia associated with an attack of tetany. The deaths that occurred in coma cannot be satisfactorily explained at present. Gross changes in body tissues were not much in evidence in any case and microscopic examinations were not routinely made. Cachexia was not much in evidence, most animals maintaining fairly constant weight or even gaining.

With the exception of no. 21 none of the animals developing an infection of any kind displayed tetany during that period. One dog suffered a postaxillary abscess at the site of a parathormone injection; another developed a large abscess on the leg at the site of an accidental subcutaneous injection of calcium lactate. Until these abscesses were drained and healed, neither of these animals developed tetany, although both had previously experienced several severe attacks and both subsequently died in tetany. None of the distemper animals showed tetany after this condition had progressed to a recognizable stage though examination of the blood showed that calcium and phosphorus concentrations were such that tetany might have been predicted.

Many investigators have reported that disturbing animals that were on the threshold of tetany induced attacks; others have failed to confirm this. There was great diversity of response among our dogs. No. 21, at one stage, could be thrown into tetany by rubbing the skin or by the procedures incident to bleeding. At other times, when examination of the blood indicated that a threshold condition existed, indeed when tetany actually occurred in a few hours, no amount of physical disturbance would induce an attack. Occasionally animals seemed to be on the verge of an attack which would be delayed by voluntary exercise. No. 36 was frequently thrown into tetany by the excitement of his efforts to mount, at command, a 3-foot operating table. The reason for this variable reaction is not apparent at present.

In this connection it is significant that most of the unobserved attacks occurred during the night, as indicated by the condition of the cage and of the animal. Some died during the night, evidently in tetany, as shown by the posture, when presumably they had been quiet for several hours. In view of the importance to be attached to the inorganic phosphorus concentration in relation to tetany, to be discussed presently, it is difficult to reconcile these latter observations with the report by Kleitman (1925) that in the human there is greater phosphorus elimination during sleep. It may be mentioned that Havard and Reay (1926) found that exercise increased and later decreased inorganic phosphemia.

The spastic condition of certain muscles sometimes observed in latent tetany was unquestionably relieved by voluntary exercise. This condition did not develop in any subjects in this series until 3 or 4 weeks after operation and then only in 5 cases.

No. 22, a young adult female, apparently nulliparous, was operated January 13, 1927, and developed tetany on the 3rd day. She was found to be in oestrus on the 39th day after operation and immediately placed with a male dog. Superficial evidence of oestrus persisted 4 days. Death occurred on the 76th day, from distemper. Autopsy revealed 5 embryos in the uterus.

Carlson (1912b) found that late pregnancy intensified the symptoms of tetany. It cannot be said that this was true of this animal. On the contrary, symptoms were less severe during this period than previously. It is probable, however, that progress would have been different after fetal calcification had begun. This animal confirms the report of Dragstedt (1924) that parathyroidectomy does not interfere with subsequent oestrus or pregnancy.

Blood mineral alterations. Beginning with no. 9, frequent determinations of plasma calcium were made on all animals. Inorganic phosphorus was determined by the method of Briggs (1922), beginning with the 49th day of observation of no. 21. Calcium was determined by the method of Collip and Clark (1925). In our hands the method proved accurate to ± 3 per cent. It is desired to emphasize further the necessity for strict adherence to the precautions outlined by these authors. At the suggestion of Tweedy (1926) we had made special centrifuge tubes[2] which were drawn to an inside diameter at the tip of 1 to 2 mm. For titrating the permanganate solution, the reservoir microburette described by Koch (1926) was used. Blood samples were drawn into a syringe containing 3 to 4 mgm. of heparin moistened with a drop of 0.9 per cent NaCl, the excess being driven out with the plunger. Determinations were made at once. The calcium oxalate precipitate was usually washed four times.

Duplicate determinations of both elements were made whenever enough blood was available. Since many dogs were small, it was deemed undesirable to draw too large amounts as it was often necessary to bleed one or more times daily for several days.

Values for calcium in normal dogs were somewhat higher than reported figures of human serum (Rosen and Krasnow, 1926), and also slightly higher than many figures reported for dogs, but closely paralleling those of Fisher and Larson (1925) and of Reed and Tweedy (1926). There was quite a wide variation between different animals but fair constancy in the same dog. Confinement under constant conditions for some time tended to reduce the concentration. Inorganic phosphorus values were likewise fairly constant, though more variable than those for calcium. A small number of determinations on normal puppies showed the phosphorus concentrations to be considerably higher than in adult dogs.

The tetany level of calcemia has been considered to be 7 mgm. per 100 cc. of serum or plasma, both in experimental animals and in human patients in post-operative tetany, spasmophilia and adult idiopathic tetany. More recently, however, there have appeared many reports showing that the tetany level is quite variable (Collip, 1925; Fisher and Larson, 1925; Salvesen, 1924; Sloan, 1926; Gross, 1926; Luckhardt, Waud and

[2] Prepared according to specifications by Central Scientific Co.

Brannon, 1926; Cameron, 1926). This variation is also apparent in clinical conditions, according to the reports of Howland and Marriott (1917), Howland and Kramer (1923), Erdheim (1926), Miraglia (1926) and of Hjort and Ader (1927). In this work it was very early apparent that when tetany in the same animal at different times, or in different animals at the same time, occurred at approximately the same level of calcemia, there was no possible correlation between this factor and the symptom complex, either in kind or degree.

It must also be considered that there are other conditions in which a profound hypocalcemia occurs without tetany, such as nephritis (Howland and Marriott, 1917; Mason, 1926), experimental and clinical jaundice (Buchbinder and Kern, 1927). Furthermore, tetany may occur at very high concentrations. Houssay (1926) reported a parathyroidectomized dog in which tetany occurred after calcemia had been increased by administration of parathyroid to 12.12 mgm. Occasionally our dogs also displayed tetany at high concentrations, though not constantly in any one case.

It is apparent, then, that some factor other than hypocalcemia is involved in the production of tetany. Consequently, simultaneous determinations of both calcium and phosphorus were undertaken. Greenwald (1911) seems to have been the first to observe retention of phosphorus after parathyroidectomy, and that inorganic phosphemia was not necessarily a measure of the degree of retention (1926). Alterations in phosphemia have also been found by Inouye (1924), Freudenberg and György (1922), Elias and Speigel (1921), Elias and Weiss (1922), Collip (1925), Hastings and Murray (1921) and Sloan (1926), and they are apparent in Salvesen's report (1924), although this author does not stress the fact. Collip found a very great increase in phosphemia in rabbits, which may possibly be correlated with the violent symptomatic response and refractoriness to treatment which these animals generally display. Howland and Kramer (1923) and Von Meysenbug (1921) have found hyperphosphemia in infantile tetany.

Simultaneous determinations of these elements in our animals established that, while tetany may occur at varying degrees of calcemia, it is practically always at such a concentration that the Ca:P ratio approximates 1 or less. In only 4 instances out of a total of 67 determinations of these elements on days when the animals showed tetany or spasticity, did symptoms occur when the ratio was found to be definitely above 1. In normal animals this ratio is generally 1.5 to 2. In some cases the change involved increased phosphemia together with hypocalcemia while in others it was mostly to be ascribed to the latter alone. In fact there were instances in which phosphemia was less than before operation, at the critical ratio. Occasionally the ratio was slightly greater than one when blood was drawn in

the morning and the animal displayed tetany later in the day. Our experience is that changes may occur with sufficient rapidity to bring the ratio to the critical point in a few hours.

At any rate it appears that when the ratio approaches 1, the animal is in a condition when tetany may be induced by relatively minor influences, or may be expected to occur spontaneously within a few hours. Spontaneous recovery from an attack did not, however, constantly increase the ratio, and then only slightly. In fact it may fall farther. On the other hand, in some cases when blood was drawn just before, or at the first sign of an attack, the ratio was found to be much less than 1. It is probable in these instances, that one or more unobserved attacks had already occurred, of which there were no visible signs. This was known to have been the case in instances observed by other persons and later reported to the authors.

It must be understood, however, that present available evidence does not warrant any claim that these findings offer an *explanation* of tetany, but are to be considered rather as an *indication* of a threshold condition of the animals.

Howland and Kramer (1923) have found that when the product Ca × P falls to between 30 and 40, rickets is likely to occur and is inevitable if it falls below 30. A similar relationship has been found by Henderson, Noble and Sandiford (1926) in cases of ununited fracture. It has been observed many times that tetany may occur in rachitic children and in experimental rickets in animals. In order to determine whether this product could be shown to bear any relation to the incidence of tetany in these animals, it was derived in every case and was found to be quite variable after definite symptoms of parathyroid deficiency had appeared. In general, perhaps, it might be said that a great decrease in the product may indicate a condition in which tetany will occur if other related conditions are fulfilled, but the relationship is most casual since some dogs showed violent tetany with a product of 50 or even 60, while others showed no symptoms when the product was below 30. In normal dogs the product was very high, ranging from 60 to more than 100. The lowest concentration of calcium without tetany was 4 mgm.; lower figures have been reported.

A possible explanation of the concurrence of rickets and tetany lies in the fact that if the calcium and phosphorus concentrations are such as to give a ratio of 1 or less, and at the same time a product below 30, both conditions will occur. This conception is well illustrated by the report of Brown, Tisdall, Courtney and McLachlan (1922) of two rachitic infants, one of which suffered tetany also. Table 2 is arranged from their published figures.

Examination of reports of calcium and phosphorus determinations in tetany by other workers shows confirmation of our findings though apparently the mathematical relationship at the critical stage was overlooked by

the authors (Hastings and Murray, 1921; Greenwald, 1926; Fisher and Larson, 1925; Salvesen, 1924). Hammett (1924) has suggested some such relationship though less definitely, merely stating that in rickets there is a decrease in the P:Ca ratio, or in the terminology of this paper, an increased Ca:P ratio, while in parathyroid deficiency the reverse is the case. Howland and Kramer (1921, 1923) and Kramer, Tisdall and Howland (1921) reported several series of cases of infantile tetany in which a similar ratio of calcium and phosphorus is shown in relation to tetanic attacks, though it must be admitted, less constantly, 5 out of 14 cases showing tetany with high ratios. However, it should be recalled that the fundamental pathology in these cases is different from that in experimental parathyroidectomy. There is also the possibility of concurrence of other tetanigenic factors known to be operative in clinical subjects. In view of the fact that M. M. Hoskins (1924) has shown that the parathyroids of rats are particularly resistant to influences that readily alter the functions of other organs, it does not seem probable that clinical functional disturbance of these glands would be likely to occur without involvement of other factors,

TABLE 2

SUBJECT	Ca	P	Ca:P	Ca × P	
B	9.2	2.8	3.28	25	Rickets only
C	5.2	6.8	0.75	35	Rickets and tetany
	10.5	5.2	2.019	54	Normal child

while in experimental conditions, such involvement would not be likely to be present before operation in any large proportion of animals and so would play no part in the subsequently produced symptom complex. For this reason, caution should be observed in drawing too close parallelism between clinical and experimental observations in this connection.

In a personal communication, Prof. A. T. Cameron suggested a point later amplified in publication, by way of explanation of the variations in the tetany level of calcemia in the same animal. Cameron and Carmichael (1926) have found that a sudden fall in barometric pressure induced tetany in thyroid-fed rats and attempted to correlate this with the seasonal incidence of tetany.

Acting upon this suggestion, records have been kept for more than a year of daily barometric changes, temperature and relative humidity. The condensed data are shown in table 3. Out of 53 observations of tetany, or of a ratio of 1 or less without tetany, the larger proportion occurred when the barometer was constant or rising. Similar figures were obtained in those animals in which only calcium determinations were made. It was at first thought that a sudden fall in barometric pressure when an animal

was in a state of latency bordering on actual tetany, might cause variations in the time of appearance of symptoms. But it is apparent from these figures that this factor is not responsible. To be sure, a large percentage of recorded barometric changes is very small, as this is remarkably constant in this locality throughout most of the year. However, exclusion of the smaller changes in favor of those of greater magnitude does not materially alter the statistical relations. It seems more probable that the explanation of the greater incidence of tetany in certain months of the year is to be found in the same factors which induce greater incidence of rickets in these same seasons, rather than in meteorologic conditions. Moro (1926) has suggested as other possible factors involved in this seasonal incidence, decreased irradiation, changes of diet and of physical activity, and increased irritability of the vegetative nervous system at certain periods.

That inadequate irradiation may be a factor is possible. Several observations have indicated that either natural or artificial irradiation

TABLE 3

Net barometric changes during preceding 24 hours

TETANY	CONSTANT OR RISING		SUDDEN FALL	
	Number	Per cent	Number	Per cent
Mild to severe	23	43.4	16	30.0
Violent	3	5.6	4	7.5
Low Ca:P ratio without tetany	5	9.4	2	3.7

improves the condition of spasmophilic patients and partially parathyroidectomized animals but does not improve that of animals subjected to complete extirpation when applied after operation (Swingle and Reingold, 1923; Bloch and Faber, 1925; Drücker and Faber, 1926; De Gennes, 1925; Grant and Gates, 1924; Grant and Goldman, 1924; Casparis and Kramer, 1923; Ferri, 1925; Myerson, Gunther and Laurens, 1925; Falkenheim and György, 1924; Lesné and Turpin, 1925; Hammett, 1924; Jones, 1926). Some definitely assign the results of irradiation in deficiency to stimulation of the parathyroids. There have been some reports of parathyroid involvement in rickets, which condition is definitely improved by irradiation. J. Erdheim (1914) has found these organs enlarged in some cases of rickets. Doyle (1925) has confirmed this observation on rachitic chickens and Nonidez and Goodale (1927) have found that in rachitic chickens there is a stage of hypertrophy and hyperplasia followed by a stage of regression. Hammett (1924) believed these changes to be secondary affects.

There seems to be no possible correlation between our records of relative

humidity and the incidence of tetany in operated animals. It has seemed that symptoms were more severe at high atmospheric temperatures, particularly if humidity was also high. But it cannot be said that attacks were any more frequent at high temperatures than otherwise. However, it must not be overlooked that nos. 8, 9, 20, 21, 23, 26 and 43, which have survived the longer periods were all operated during the coldest periods occurring in this locality during the time over which these observations extend. Whether this is of more than incidental significance cannot now be stated.

Treatment. Since we were not primarily concerned with methods of treatment, there is little to offer in this connection except confirmation of the results of others. Whenever diarrhea appeared, lactose was added to the diet. Our observations are too few to permit of concluding that the invariable disappearance of this symptom was due to this treatment. Voluntary exercise invariably relieved spasticity but this may have been a general rather than a specific effect. A generous supply of parathyrin (Parathormone Lilly) was placed at our disposal early in the fall of 1926.[3] Whenever this was available, it was used for treatment, otherwise calcium lactate was used. In only two instances were symptoms of parathyrin overdosage produced as shown in the following protocols.

No. 21, male, 13.6 K., on the 5th day after operation went into severe tetany at 9:30 a.m. and received 3 cc. parathormone subcutaneously. Symptoms abated in 30 minutes and he lay quietly in the cage until 12:00 m., when he suddenly experienced a violent convulsive siezure of about 5 minutes' duration and seemed greatly depressed during the day. This was not at all typical of tetany. A blood sample taken during the seizure was lost.

No. 23, female, 13.6 K., went into tetany for the first time at 8:30 a.m. on the 3rd day after operation. Ca, 5.78; P. 8.85; Ca : P ratio, 0.653. After bleeding 3 cc. parathormone were given intravenously at 9:00 a.m. At 11:00 she voluntarily arose and moved about freely, though the gait was very uncertain and the legs seemed weak. Some tremors were apparent still at 12:00. At 3:00 p.m. she lay quiet in the cage but when aroused moved about in a normal manner. At this time Ca, 9.29; P, 10.7; Ca:P ratio, 0.676. At 5:00 she was sleeping and was very listless when aroused. The heart beat was slow and strong. There was no evidence of tetany. On the following morning she was found dead, curled up as if asleep. There was no evidence of tetany, diarrhea or vomiting. Autopsy showed no gross changes in the tissues.

While not presenting the entire symptom complex of overdosage described by Collip, it seems likely that this was responsible for the behavior of both these animals.

Our experiments are being continued with a view to obtaining further information on the behavior of the Ca:P ratio under modified conditions, and on changes in the diffusible and non-diffusible fractions of both calcium

[3] This was supplied by courtesy of Dr. J. H. Warvel, Medical Director of Eli Lilly & Co.

and phosphorus, in the hope of throwing further light on some of the complicated problems indicated in the above discussion.

SUMMARY

1. Observations on 35 parathyroidectomized dogs are reported and the data discussed with relation to similar experiments carried out by other investigators.

2. The complete symptom picture is somewhat modified as compared to large series of observations reported from other geographical regions.

3. Laryngospasm was the most common symptom observed in puppies.

4. Dogs are more susceptible to infections after parathyroidectomy, in confirmation of other reports.

5. Animals suffering infection of any kind, with one exception, did not display tetany during the period of persistence of the complicating condition.

6. Parathyroidectomy did not prevent subsequent oestrus and pregnancy. Early pregnancy did not aggravate the symptoms of tetany in the one case observed.

7. The tetany level of calcemia is not constant in different animals nor in the same animal at different times.

8. Tetany practically always occurs if the proportions of calcium and phosphorus in the plasma are such that the ratio Ca:P is 1 or less.

9. The product Ca $\times$ P bears no relation to the incidence of tetany.

10. There was no relation between the severity or type of symptoms and the Ca:P ratio, or the absolute concentration of either element at the critical ratio.

11. Observations for more than a year have failed to show any relation between depression of barometric pressure and the incidence of tetany.

12. The results of treatment with calcium lactate and parathyroid extract confirm the work of others.

13. Changes in mineral content of the blood do not account for all the observed facts.

14. *These* experiments do not show that there is any pronounced modification in the incidence of parathyroid tetany in dogs, peculiar to this geographical region.

Thanks are due Dr. F. T. Rogers for constructive criticism of the work and suggestions in the preparation of the manuscript; to Dr. C. G. Duncan for the histological work; to Dr. J. H. Warvel and Eli Lilly & Co. for the supply of Parathormone and for valuable suggestions; and to many other colleagues in this and other institutions who have contributed suggestions and assistance that materially facilitated the progress of the investigation.

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NOTE: At the time of preparation of this manuscript we overlooked a report by Gates and Grant (1927) in which it was clearly shown that in parathyroidectomized rabbits the Ca:P ratio was lowered as described in this paper and at the tetany level was less than 1. This paper confirms their report.

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STUDIES ON THE KNEE JERK

V. The Effect of Hunger Contractions upon the Knee Jerk[1]

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Several years ago one of us (1) studied this problem in the course of a general investigation on the mechanism of hunger. Although the work on this particular phase seemed complete at the time, newer methods of eliciting and recording the knee jerk continuously over prolonged periods of time warranted the reinvestigation of the problem.

Methods. In a previous paper of this series (2) one of us described a knee jerk apparatus by which it is possible to deliver constant blows to the patellar tendon at a definite interval for long periods of time and quantitatively record the responses produced. By means of this apparatus, and by using the balloon method of recording gastric motility we were able to take simultaneous records of the gastric tonus and hunger contractions and of the knee jerks over the period of time necessary for the experiment. The time of an experiment never lasted more than three hours, since it is very fatiguing for a subject to sit perfectly still for a longer period, and we thought the uneasiness produced might influence the results. The interval between stimuli to the patellar tendon was made eight seconds, since this interval had been found in previous work to cause no measurable fatigue in the knee jerk mechanism. Also by using an interval as short as this, we were able to record changes in the knee jerk of short duration. Records were taken during a gastric quiescent period, during a hunger period, and during the following quiescent period.

Results. During a hunger period there is a general persistent rise in the knee jerk ranging from one hundred to several hundred per cent above the quiescent period, depending upon individual differences in subjects (fig. 1 *B*). In addition to this, there is an increase in the knee jerks during the individual hunger contractions ranging from six hundred to one thousand per cent. This marked increase of the knee jerk almost invariably occurred at the down stroke of the writing lever (that is, at the end of the individual contractions) (fig. 1 *C*). Always at the end of the hunger

[1] This work was conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

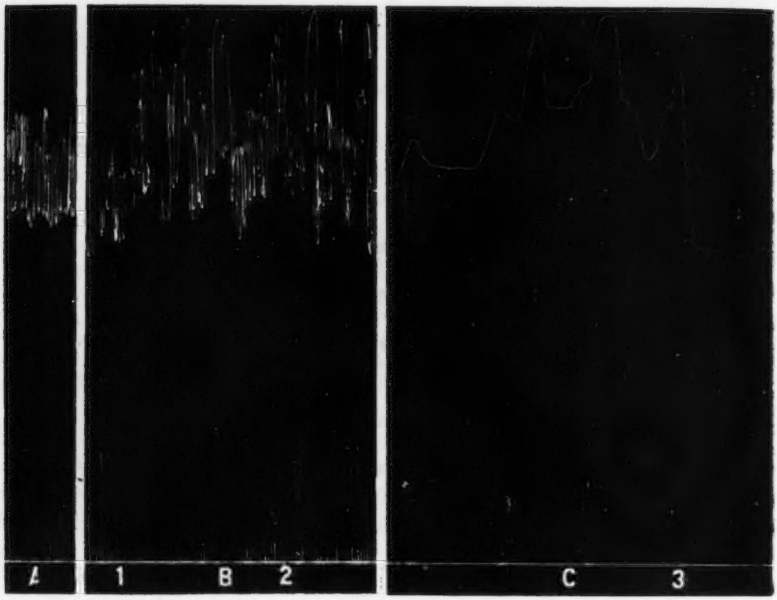

Fig. 1. Man: Lower record, knee jerk. Upper tracing, gastric tonus and hunger contractions. *A*, gastric quiescence. *B*, beginning of period of gastric hunger contractions felt at *2*) and parallel increase in knee jerk. *C*, gastric hunger motility and knee jerk recorded on faster moving drum, showing the generally higher knee jerk on fundus relaxation, *3*.

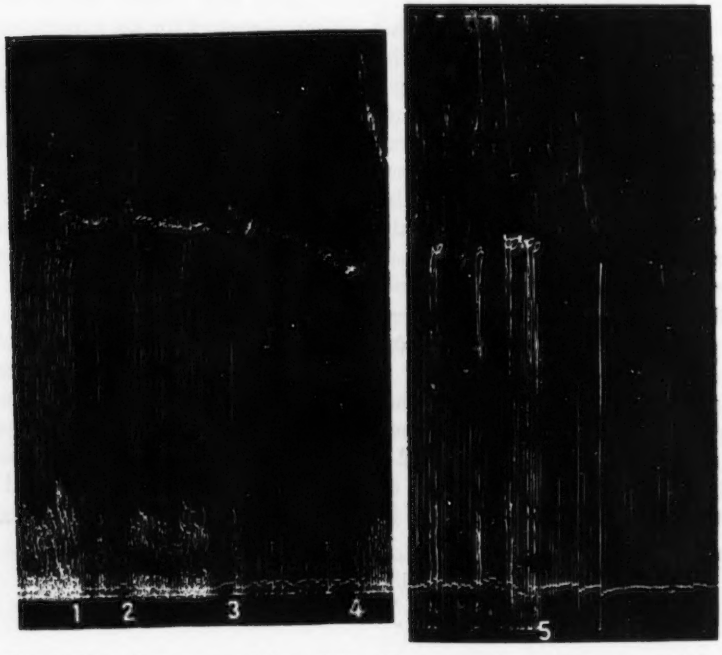

Fig. 2. Man: Lower record, knee jerk. Upper record, gastric tonus and hunger contractions. *1*, end of a hunger contraction period. *2*, *3*, Borborygmi and flatus. *4*, inflation of gastric balloon. *5*, end of gastric hunger contraction period.

period the amplitude of the knee jerk decreased very rapidly. In addition to the above results, it was found that the occurrence of borborygmi, the passage of flatus, of inflation of the balloon during a quiescent period produced a very marked rise in the knee jerk amounting to several hundred per cent (fig. 2).

SUMMARY

By the new and more quantitative method of eliciting the knee jerk the previous work showing an increased amplitude of the knee jerk during a hunger period, as well as during individual hunger contractions are confirmed. Borborygmi and passage of flatus also augment the knee jerk.

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THE EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT

IV. Length of Sexual Life[1]

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We have previously discussed the effect of various amounts of sexual indulgence on growth, spontaneous activity and food consumption (Slonaker, 1927 a, b, c). In the first of these papers we gave in detail the plan and scope of the experiment. Only a brief summary, therefore, will be given here.

The 126 pure bred albino rats used were divided into five groups. Group A, consisting of strict abstainers in which no sexual intercourse was permitted during their entire life span, contained six bachelors and twenty-nine virgins. Group B, consisting of 4 males and 9 females, were light breeders. The females of this group delivered not less than two nor more than three litters each during their life span. Group C, composed of 4 males and 9 females, was classed as medium breeders. That is, the mothers nursed their young until weaned at 20 days after which rebreeding occurred as soon and as often as possible throughout life. Group D, heavy breeders, contained 4 males and 9 females. In this group the young were destroyed after weighing at birth and the females rebred at the earliest possible date and as often as they were able to reproduce. Group E consisted of animals which had excessive coitus without reproduction. This group was composed of 12 normal males and 40 hysterectomized females. They were distributed 3 males and 10 females to a large cage and were continually together during their life span. Since hysterectomy did not interfere in any way with the normal oestrual cycles, excessive sexual intercourse took place without the ability to bear young.

Five females from each of the groups A, B, C and D were kept in individual revolving cages and all others were confined in stationary cages. All were kept in the same room, in the same environment and fed the same well-balanced synthetic food. Any differences in the results we attribute to the amount of sexual indulgence permitted.

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and the Committee for Research on Sex Problems of the National Research Council.

Since the experiment was started at the average age of 74 days, before few, if any, of the animals had reached sexual maturity, we were able to determine the length of the period during which they were sexually active. In groups C and D this was done by true matings and delivery of young as well as by activity of those in revolving cages. In groups A and E which delivered no young and in group B whose limited number of litters were mostly born early in life the mating test could not be made. We have previously shown (Slonaker, 1924) that the rhythmic recurrence of oestrus coincides with marked rhythmic increases in voluntary activity and that when an oestrus fails to occur the corresponding spurt of activity is also wanting. It was also demonstrated that the rhythmic spurts of activity

TABLE 1

Giving the average age in days of each group for various stages of sexual life as indicated and the length in days of sexual life

GROUP	NUMBER	AT PUBERTY	AT LAST REGULAR OESTRUS	AT LAST OESTRUS	AT LAST PRODUCTIVE OESTRUS	PUBERTY TO LAST REGULAR OESTRUS	PUBERTY TO LAST PRODUCTIVE OESTRUS	PUBERTY TO LAST OESTRUS	AT FIRST LITTER	AT LAST LITTER	AVERAGE NUMBER OF LITTERS
Rotary cages											
A	5	73	627	836	?	554	?	763			
B	5	93	659	728	?	566	?	635	159	395	2.60
C	5	105	657	795	519	552	414	690	177	541	6.60
D	5	86	697	771	399	611	313	685	147	421	6.60
Stationary cages											
B	4	93	?	?	506	?	413	?	115	528	2.75
	3	91	?	?	635	?	544	?	113	657	3.00
C	4	94	?	?	619	?	525	?	116	641	11.50
D	4	92	?	?	434	?	342	?	114	456	11.75

were not manifested before puberty nor after the menopause. Making use of these data we were able to determine the length of sexual life of only those animals which spent their lives in revolving cages. Owing to the fact that group E spent the whole life span in stationary cages with males and were unable to bear young we were unable to obtain the length of sexual life of this group.

In table 1 we have given average results obtained for the different groups. In all the animals kept in revolving cages the age of puberty was determined by the first rhythmic increase in spontaneous activity. This sometimes is not a correct test owing to the fact that some animals are slow in learning to turn the cages. This appears to have been the case in group C in which only one showed the average normal age at sexual maturity.

With the animals kept in stationary cages which were mated when the females were at the average age of 75 days the age of sexual maturity was ascertained by subtracting the average gestation period of 22 days from the age at delivery of the first litter. If we compare the average age at puberty of all females in revolving cages, as determined by activity, with that of those in stationary cages gotten by breeding we find a slight discrepancy. That is, sexual maturity, as indicated by the first slight rhythmic increase of activity, occurred about four days before the first oestrus as determined by reproduction. This indicates that the first oestrus is less likely to be productive in mating than the second.

In our former paper (Slonaker, 1924) we showed that there was a marked variation in the cessation of the oestrual cycles at the menopause. Some individuals had regular cycles and stopped abruptly without further evidence of cycles while others had periods, during which no cycles occurred, of several days or weeks interspersed between intermittent cycles varying from one to six or seven. These periods of cessation became longer and longer and the number of cycles fewer in number until they finally stopped. In the table we have given for the females in revolving cages the average ages of each group both at the time the rhythmic cycles stopped and when the last cycle was noted, as indicated by their activity curves. In the case of the females which were continually with their mates in stationary cages we were able to determine only the last productive oestrus by subtracting the average gestation period from the age of delivery of the last litter. In these animals we made no tests to determine if later cycles occurred. If they did occur and resulted in coitus they were non-productive. A large number of mating tests were made on the females of groups B, C and D in revolving cages after the cessation of the regular cycles. Most of these trials were negative, but in a few cases coitus occurred as indicated by the presence of sperm in the smear. Such cases, however, were not productive of litters, but resulted in pseudo-pregnancy. The oldest recorded age at which coitus took place was 681 days.

If, as we have assumed, the rhythmic increases in spontaneous activity which are contemporaneous with the oestrual cycles are due to the follicular hormone, then this hormone must be still active at an age when the females are no longer able to breed. We are unable to state whether the inability to bear young was due to a lack of ovulation, to a depleted condition of ova rendering them incapable of fertilization, an inability to implant fertilized ova, or to some other cause. Indications point to at least a partial maturing of Graafian follicles sufficient to stimulate activity and changes in the vaginal mucosa, but generally insufficient to excite coitus. This agrees with our former results (Slonaker, 1927d) in which we found cornified cells in vaginal smears of old females which had passed the menopause. We concluded that changes in the vaginal mucosa could be

induced by smaller amounts of the follicular hormone than that required to incite coitus.

In table 1 we have given the average length of sexual life for the different groups. In groups A, B, C and D in the revolving cages it is given for the period from puberty to the cessation of regular oestruation and also from puberty to the last irregular oestrus as indicated by the activity curves and vaginal smears. In groups B, C and D in the stationary cages the length of the sexual life is from the first productive oestrus to the last productive oestrus. In group B (stationary cages) the averages are given for the four females constituting the group and also for three of this group which were bred a third time at the average age of 544 days. One of this group was only bred twice which occurred early in life and was not tested again until late in life, when the results were all negative.

The average length of sexual life, as indicated by regular cycles of activity, for the different groups in rotary cages was almost the same for the virgins (A), the light breeders (B), and the medium breeders (C). It was nearly two months longer for the heavy breeders (D). The medium breeders showed the shortest duration, but this may have been due to the fact that this group was slow in learning to turn their cages which resulted in the first cycle appearing some fifteen days later than the average age of puberty. If allowance be made for this late start the order for the length of sexual life for these groups from shortest to longest would be A, B, C and D. If true active sexual life is determined by the ability to bear young and not by rhythmic oestrual cycles, as indicated both by activity and vaginal smears, then these values given in table 1 for the length of sexual life are too long. Many mating tests were made with these animals at various times before the cessation of their regular cycles and in only one case did we get productive matings after the age of 600 days. In the later part of sexual life most cases of successful coitus, even though insemination occurred, resulted in pseudopregnancy. Since our mating tests were made but once each day and the receptivity of the female is of short duration many potential productive cycles may have been missed. The last productive oestrus which we obtained in these groups is therefore most probably at an earlier age than it should be because of failure in placing the male and female together at the critical time.

In the group kept with mates in stationary cages in which the ages of sexual maturity and cessation of sexual life were determined by productive matings we find that the length of sexual life was much shorter than that gotten by smears, activity records and mating tests of the animals in rotary cages. This indicates further that cycles late in life of the rat are frequently non-productive, even though they may often incite coitus. It is interesting to note that the three light breeders which were bred for a third litter late in life had a productive oestrus later in life than either group C or

group D. It is also seen that group C had a longer sexual life than group D. This was also true of the C and D groups in the revolving cages. These results lead us to conclude that light breeding in which two or three litters are born is conducive to a longer sexual life than no breeding, as well as heavier breeding. The data also indicate that the rearing of the young is not so harmful and influences the length of sexual life less than destroying the young at birth.

Pseudopregnancy. In a former paper (Slonaker, 1925) we showed that many successful copulations, as indicated by the presence of sperm in the vaginal smears, failed to result in gestation and the delivery of young. The activity curve and the lack of oestrus was typical of true gestation for from 11 to 15 days after which rhythmic cycles began. Making use of these data we were able to calculate the per cent of pseudopregnancies re-

TABLE 2

Giving the average age distribution of total productive copulations of groups in stationary and in revolving cages, and of total non-productive copulations in revolving cages

AGES	GROUP B			GROUP C			GROUP D		
	Per cent of total productive		Per cent of total non-productive	Per cent of total productive		Per cent of total non-productive	Per cent of total productive		Per cent of total non-productive
	Stationary	Revolving	Revolving	Stationary	Revolving	Revolving	Stationary	Revolving	Revolving
Puberty to 250 days...	73	69	70	30	37	34	49	53	20
250 to 500 days.....		31	20	44	50	41	49	38	51
500 to 700 days.....	27		10	26	13	25	2	9	29

sulting from successful copulations in the different groups in the rotary cages. We found that in the light breeders, group B, 43 per cent of the copulations resulted in pseudopregnancy; in the medium breeders, group C, 50 per cent were pseudopregnant; and in the heavy breeders, group D, 66 per cent of the true matings terminated in pseudopregnancy. The average age at the last coitus in group B was 431 days; in group C, 588 days; in group D, it was 638 days. Group B was purposely not mated after the first two or three litters and the later trials were unsuccessful.

In table 2 we have given the percentage distribution according to age of the matings which resulted in pregnancy or in pseudopregnancy for the animals in rotary cages and for the productive copulations for the animals in stationary cages. In all the females composing group B, which delivered two or three litters, the first two litters were born early in life and when a third litter was born mating took place late in life as indicated. That is,

71 per cent of the productive matings occurred before the age of 250 days and the remaining 29 per cent after this age. There was a long lapse of time intervening during which no attempted matings were made. It is also interesting to note that of the total number of non-productive matings 70 per cent were in the early part of life. In group C, which reared as many litters as possible, we find that the percentage distributions of the productive matings are quite similar in both stationary and revolving cage animals. The differences we attribute to the lack of making our mating tests of the animals in rotary cages at the critical time of oestrus. The averages of all the females in the C group show that approximately 34 per cent of all productive matings occurred before the age of 250 days, 47 per cent between 250 and 500 days and 19 per cent after 500 days of age. The non-productive matings in the rotary cages were distributed in almost the same proportions. In group D, whose young were destroyed at birth and the females remated at the earliest possible date, we find a decided shift of productive matings toward early life and of non-productive copulations toward the middle and the later part of sexual life. Of all the productive matings approximately 51 per cent occurred before the age of 250 days, 43 per cent between the ages of 250 and 500 days and 6 per cent after 500 days. This further indicates that the destruction of young at birth has an inhibiting effect on reproduction late in sexual life. Since groups C and D produced almost the same number of litters it appears that the females are able to bear young only a certain number of times and if the young are destroyed at birth this number of litters will be born earlier in life.

The range in ages at last productive mating was quite variable. In the B group they were for the rotary cage animals 238 days to 457 days and for the stationary cages 121 to 672 days. The C group rotary were 383 to 607 days and the stationary 582 to 663 days. In group D in the revolving cages the ages were from 305 to 601 days and in the stationary cages from 386 to 509 days. The fact that the animals in this last group in rotary cages which delivered an average of 6.6 litters had a productive mating almost 100 days later than the stationary cage rats, which delivered an average of 11.75 litters, again indicates the inability of producing beyond a certain number of litters, even though the age is favorable.

In order to determine which sex became sterile first the twelve males and twelve females constituting groups B, C and D in the stationary cages were remated and kept with vigorous animals in the prime of sexual life. This test was made for the period from 575 days to 685 days of age. Before this latter age all ability to reproduce had disappeared. The results were as follows: In the light breeders, group B, three of the four males proved fertile, siring but one litter each at the ages of 586, 580 and 578 days. The other proved sterile after the age of 575 days. Three of the four females

were fertile giving birth to one litter each which were the results of matings at the ages of 591, 641 and 672 days. The other female was sterile. That is, 75 per cent of the males were fertile up to the average age of 581 days and 75 per cent of the females were fertile until the average age of 635 days. In group C two of the four males sired one litter each at the age of 609 days. The other two males were sterile. All of the females proved fertile, one giving birth to two litters. The ages at the last productive matings of these females were 603, 623, 651 and 663 days. That is, 50 per cent of the males were fertile until the average age of 609 days and 100 per cent of the females were fertile up to the average age of 635 days. In the group of heavy breeders, D, two of the males proved fertile, siring one litter each at the ages of 591 days. The other two males were sterile. All of the females were found to be sterile. That is, 50 per cent of the males were fertile at the average age of 591 days and 100 per cent of the females were sterile between the ages of 575 and 685 days. The last productive matings occurred at the average age of 456 days. The results of this group show that when the young were destroyed at birth in heavy breeding the females were more affected than the males; that 100 per cent of the females were rendered incapable of bearing young after the average age of 456 days while but 50 per cent of the males were so affected up to the age of 591 days. This seems to indicate further that the number of litters born is a greater factor in determining the length of productive sexual life of the female than the age of the individual. So far as these results go they show that the different amounts of sexual indulgence permitted in these different groups had no striking effect on the males except in the light breeders in which fertility was maintained by a greater number for a longer period. The effect on the females, however, was very pronounced especially in the medium breeders and the heavy breeders. In the former the period of fertility was lengthened while in the latter it was much shortened. The effect of light breeding on the females of group B approached closely that of the medium (normal) breeding group.

SUMMARY

In regard to the effect of sexual abstinence, light breeding, medium breeding and heavy breeding on the length of the sexual life the following conclusions may be drawn from the described results:

1. The first productive oestrus was found to occur at a later age than the first oestrus demonstrated by smears and activity tests.

2. The last productive matings occurred at an earlier age than the last oestrus as indicated by vaginal smears and activity curves.

3. The amounts of sexual activity permitted the different groups in the rotary cages increased the length of sexual life of group D but did not affect

the other groups to any great extent. In no case was the amount of sexual activity much greater than half the possible amount. The rhythmic cycles of the virgins ceased at an earlier age than in any of the other groups.

4. In the animals in stationary cages the length of productive sexual life from the shortest to the longest was group D, 342 days, group C 525 days, and group B 544 days.

5. The removal of the young at birth, followed by prompt remating of the mother, caused a shifting of reproduction toward early life.

6. The number of litters born appears to have been as great a factor as age in determining the length of reproductive sexual activity.

7. Of the whole number of copulations which took place in the groups in rotary cages only 57 per cent in the B group, 50 per cent in the C group and 34 per cent in the D group resulted in pregnancy and the delivery of young. All other copulations resulted in pseudopregnancy.

8. Fertility tests showed that in group B 75 per cent of the males were fertile at 581 days and 75 per cent of the females were fertile at 635 days; in group C 50 per cent of the males at 609 days and 100 per cent of the females at 635 days were fertile; in group D 50 per cent of the males were fertile at 591 days and 100 per cent of the females had their last productive mating at the age of 456 days.

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THE INFLUENCE OF YEAST IN THE DIET OF THE RAT UPON THE ACTIVITY OF ISOLATED SEGMENTS OF DUODENUM AND COLON

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Young healthy albino rats were selected and divided into four groups, rats in each group being similar as to age and sex.

The basal diet consisted of purified foodstuffs free from vitamin B as prepared by the Harris Laboratories. Fleischmann's yeast, dried at room temperature, supplemented this diet.

BASAL DIET		YEAST SUPPLEMENT
Food	Per cent by weight	
Starch	54	Group I. Basal diet only
Casein	18	Group II. Basal diet plus 1 per cent by weight yeast
Lard	16	Group III. Basal diet plus 10 per cent by weight yeast
Butter fat	8	Group IV. Basal diet plus 25 per cent by weight yeast
Complete inorganic salt mixture	4	
Cod liver oil 2 to 4 drops daily per rat		
Lemon juice 2 to 4 drops daily per rat		

After 14 days on diets motility experiments were begun. Experiments were conducted thereafter upon 30 rats subjected to the above diets for varying numbers of days up to 77. Four segments were compared simultaneously.

Animals were decapitated and strips of duodenum immediately below the stomach and of colon and rectum as low as possible excised at once and placed upon cotton wet with Locke's solution at 0°C. Two strips of equal length of duodenum and of colon from each rat were suspended in warm oxygenated Locke's solution and arranged in connection with light recording levers of equal weight and length. The strips not used immediately were kept at 0°C. and records made the succeeding day.

The graphic records were compared as to frequency and amplitude of contraction and duration of activity of the segments.

The figures indicate the number of experiments in which segments from rats fed diets containing no yeast or 1 per cent of yeast were greater, less or equal in activity to those from rats fed diets higher in yeast content.

COMPARISONS	FREQUENCY OF CONTRACTION			DURATION OF CONTRACTION			AMPLITUDE OF CONTRACTION		
	Greater	Less	Equal	Greater	Less	Equal	Greater	Less	Equal
No yeast vs. 10 per cent yeast	1		1	1		1	1		
1 per cent yeast vs. 10 per cent yeast	5	2	4	3		4		6	5
No yeast vs. 25 per cent yeast		2	2			2			2
10 per cent yeast vs. 25 per cent yeast	1			1					1
Totals	7	4	7	5		7	1	6	8

Segments from rats fed diets containing no yeast or low in yeast total greater activity in 13 experiments, less in 10, and equal in 22.

The number of days on diet is a factor in these experiments because rats fed diets containing no yeast become greatly debilitated through lack of vitamin B. Before the stage of debility segments from such rats show hyperactivity; and later hypoactivity. The two tend to balance in the above table making rats fed 1 per cent yeast more dependable for comparison.

CONCLUSION

Comparison of records and direct observation of contractions of isolated segments of rats' intestine do not indicate increased motor activity as a result of diets high in yeast.

THE INFLUENCE OF CEREBRAL BLOOD-FLOW ON RESPIRATION[1]

I. The Respiratory Responses to Changes in Cerebral Blood-Flow

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1. Introduction and methods. In studying the influence of morphine and other drugs on respiration, Harer and I (1), (2) found that the usual response of the respiratory center to an alteration in general blood pressure might be reversed when the center is markedly depressed. It seemed possible that the implications of this observation, if established by experiment, would have significant bearing upon our ideas of the regulation of respiration, and the work which is described in this and the following papers was undertaken for the purpose of identifying and testing those implications. The conclusions to which the work has led have been combined into a general conception of the interplay of the most important factors in respiratory control which is similar to that recently developed by Gesell (3), (4). This conception represents the elaboration of a tentative hypothesis formulated early in the work as a result of suggestions contained in an essay by Pearce (5), in which emphasis was placed upon the dependence of pulmonary ventilation upon CO_2 pressure within the cells of the respiratory center and upon rate of blood-flow through it. Since this hypothesis has served as a guide in designing experiments, it may properly be stated at the outset of this account.

The chief element in the chemical regulation of respiration is the concentration of stimulant material within the cells of the respiratory center. This is dependent upon a balance among three factors: Concentration of stimulant material in arterial blood; rate at which stimulant material is produced within the cells of the center; rate of blood-flow through the center, which determines the extent to which stimulant material can accumulate there.

Our work was concerned largely with the second and third items in

[1] Preliminary reports of the work detailed in the following papers were published in the Proceedings of the Physiological Society of Philadelphia (Amer. Journ. Med. Sci., 1926, clxxii, pp. 152 and 926) and of the American Society for Pharmacology (Journ. Pharm. Exper. Therap., 1927, xxxi, 219).

this schema because in the development of respiratory physiology (until the work of Gesell) these have been studied least. In this paper an account is given of methods used in the study of cerebral blood-flow, of influences by which it may be altered, and of the respiratory responses to such alteration.

Approved methods for studying blood-flow through a single organ include measurement and control of arterial inflow, measurement of venous outflow, and perfusion of the organ, isolated or in situ. The application of any of these methods to the brain is attended with peculiar difficulties. These are greatly increased in experiments such as these, in which it is required that natural respiration be maintained and in which it is therefore not permissible to open the chest.

Efforts to measure accurately or to control inflow of arterial blood to the brain are partially frustrated by the inaccessibility of the anterior spinal artery. We have not succeeded in finding a way of occluding this, and hence it has not been possible to divert all of the cerebral blood supply through an accessible artery in such fashion that it could be measured by means of a stromuhr or Venturi meter. Furthermore, there is an important anastomosis between internal carotid and internal maxillary arteries, as a result of which blood directed toward the brain via internal carotid may reach the distribution of the external carotid, and, vice versa, external carotid blood may enter the Circle of Willis via the internal carotid. Small muscular branches of the vertebral arteries also exist. Measurement of cerebral venous outflow is likewise rendered inaccurate by communication of the cerebral sinuses with the azygos system by way of spinal-intervertebral-intercostal anastomoses.

Perfusion of the cerebral arteries has been found to be the most advantageous of the three methods mentioned. If blood is driven into the vertebral arteries at a higher pressure than that which obtains in the animal's aorta it may be assumed that the anterior spinal artery will not add materially to the volume of blood reaching the brain, and if adrenalin be added to the blood in the perfusion system effective closure of extracranial vessels can be produced.

While all three methods have been used, after we had perfected them to the extent of our ability, we place the greatest reliance on the results of the perfusion method, and are using those obtained by the other two only in experiments in which accuracy was not essential or in which confirmatory data were required.

A descriptive outline of the three methods as we have used them follows. Since it was essential for our purpose to preserve the animal's natural respiration, it was necessary to expose the vessels involved in the preparation without opening the thorax. In dogs and cats all the great vessels—both arteries and veins—which are directly concerned in maintaining the

cerebral circulation can be clearly exposed by dissection behind the pleurae in the mediastinal space, without resection of the sternum. The most difficult to isolate is the right vertebral vein, which in all dogs and about 50 per cent of cats enters the superior cava about midway between azygos and innominate. By means of injection experiments, we have found that this vein is the most direct channel for return of blood from the brain stem in dogs, and that the left vertebral vein, which is more accessible, apparently does not communicate directly with the cerebrospinal sinuses; gas analyses of blood collected from the two vertebral veins and from the torcular Herophili confirms the inference that the left vertebral vein does not drain the central nervous system in dogs. In cats injections and blood gas analyses show that both vertebral veins communicate directly with the torcular system.

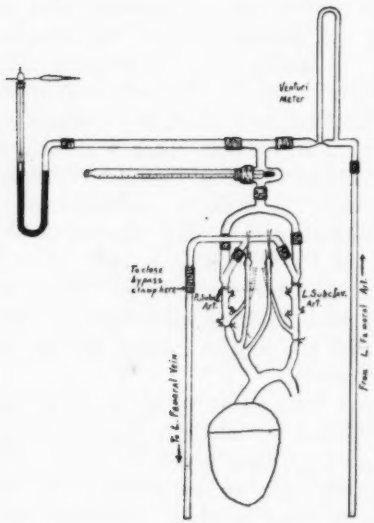

Fig. 1

The animals were anesthetized with barbital or urethane or were decerebrated by the ligature method (6). The superior caval system was isolated by dissection along the jugular and subclavian veins; having exposed the subclavian arteries, all their branches except the vertebral arteries were ligated and cut. Clotting was prevented either by heparin alone (0.1 gram per kilo by vein) or by a combination of defibrination and heparin. In dogs, three bleedings, with defibrination and reinjection, plus injection of 0.03 gram of heparin per kilo, is effective. Respiration was recorded by the Lieb-Cushny plethysmograph (7).

a. Method of measurement of cerebral arterial inflow (fig. 1). A Venturi meter (8) was connected by cannula to a femoral or common carotid artery, and its distal end was connected in turn, by a Y-tube, to the central stump of the subclavian arteries, all branches of which had been tied except the vertebrals. It was necessary only to close the carotids in order that all blood, except that of the anterior spinal artery, should pass through the meter on its way to the brain. The cannulae in the subclavian arteries were each equipped with a by-pass and these were connected by glass and rubber tubing with a femoral vein. By this means a circulation of blood through the measuring system was established before connections

with the cerebral circulation were opened. Thus it was possible to avoid driving stagnant blood or other fluid, used to fill the system, directly through the cerebral vessels at the beginning of the experiment.

We regard this method as least reliable of the three, because a considerable but unmeasurable portion of the blood measured by the meter must reach the external carotid distribution when the common carotids are closed.

b. Method of measurement of cerebral venous outflow (fig. 2). This is essentially an adaptation of Cushny and Lambie's (9) modification of Barcroft and Brodie's (10) method of measuring blood-flow through the kidney. Both subclavian arteries and all their branches except the vertebrals, and both external jugular veins, were tied. A pneumatic clamp was adjusted upon the superior cava proximal to the entrance of the vertebral veins. A tube for collecting cerebral venous blood was introduced into the left innominate vein through an opening in the external jugular, and another, for measuring pressure in the superior cava, was similarly introduced into the right innominate vein. By closing the superior cava with the pneumatic clamp, simultaneously removing the clip on the collecting tube, the time required for a measured volume (1–2 cc. in cats, 2–5 cc. in dogs) of blood to issue from the cerebral veins was determined. The level of the collecting tube was so adjusted that the pressure against which collection was made was exactly the same as the existing pressure in the superior cava.

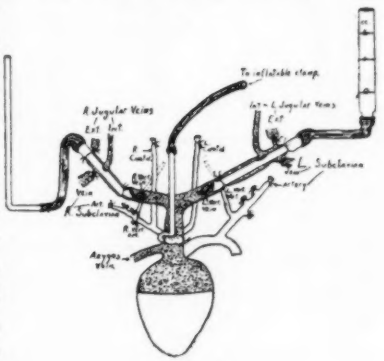

Fig. 2

An alternative source of collection of cerebral venous outflow during occlusion of the superior cava is the torcular Herophili, using a metal cannula inserted into it. In this case it is unnecessary to ligate any veins in the chest except one subclavian used in measurement of superior caval pressure.

c. Method of perfusion of the brain. Preparation of the animal is exactly the same as that described for measurement of cerebral arterial inflow. The Richards-Drinker (11) perfusion pump was used in the manner described by Richards and Plant (12) for perfusion of the kidney *in situ*. The intake of the pump was supplied with blood from a femoral or carotid artery and its output was delivered into the vertebral arteries through the central stumps of the subclavians. A by-pass was connected with a femoral vein so that circulation through the apparatus could be established

before the pumped blood was allowed to reach the central nervous system. Drugs were added directly to the blood in the intake reservoir.

II. FACTORS WHICH INFLUENCE CEREBRAL BLOOD-FLOW. *a. Changes in systemic blood pressure.* That cerebral blood-flow depends directly upon aortic blood pressure has been well established by the work of many investigators, notably Knoll (13), Gaertner and Wagner (14), Bayliss and Hill (15) and Hill and Macleod (16). In the commonly used laboratory animals the vasoconstrictor nerve supply to cerebral vessels appears to be either lacking completely or so scanty that a rise in blood pressure produced by vasoconstriction is associated with passive distention of cerebral vessels and increased cerebral blood-flow (17).

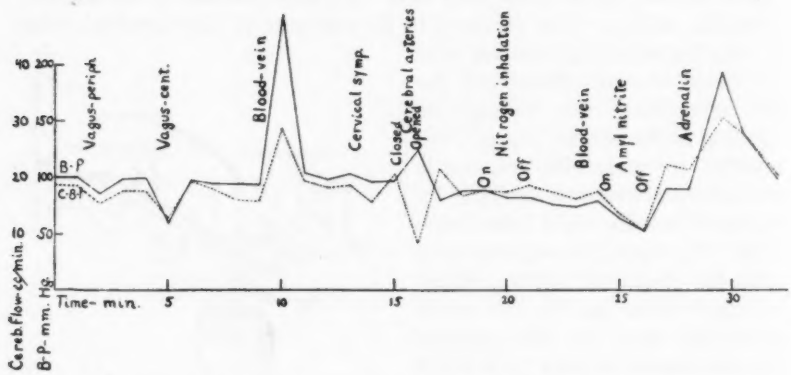

Fig. 3. Behavior of cerebral venous outflow in relation to changes in systemic pressure.

Cat, 5.2 kilos: February 23, 1925: barbital. Cerebral venous outflow by jugular method. Vertebral and internal carotid arteries open, all other branches of carotids and subclavians ligated.

We have had many opportunities for testing the truth of these statements. The results being wholly confirmatory it is sufficient to give a list of agents employed together with results of one illustrative experiment (fig. 3). The venous outflow method was used, in cats and dogs. Systemic pressure was altered by clamping and release of the abdominal aorta; by stimulation of vagus (cardiac end), splanchnic, anterior crural, and cervical sympathetic nerves; by inhalation of CO_2 or nitrogen, or by rebreathing; by hemorrhage and by infusion of blood, saline, or gum saline; by exhibition of adrenalin, pituitrin, ephedrine, amyl nitrite, and nitroglycerine.

The only exceptions to the rule that cerebral blood-flow follows passively a change in systemic pressure were an occasional slight increase in cerebral

flow in the early stages of amyl nitrite action. When blood pressure had fallen definitely cerebral flow was always decreased. Pituitrin was tried in four experiments: it produced only acceleration of cerebral blood-flow in each case. No other drugs were used which would be expected to affect blood vessels directly.

b. Vasomotor influences. In 22 tests on 7 cats no significant decrease in cerebral venous outflow resulted from stimulation of the cervical sympathetic or the central stump of a cut vagus. Adrenalin injections and

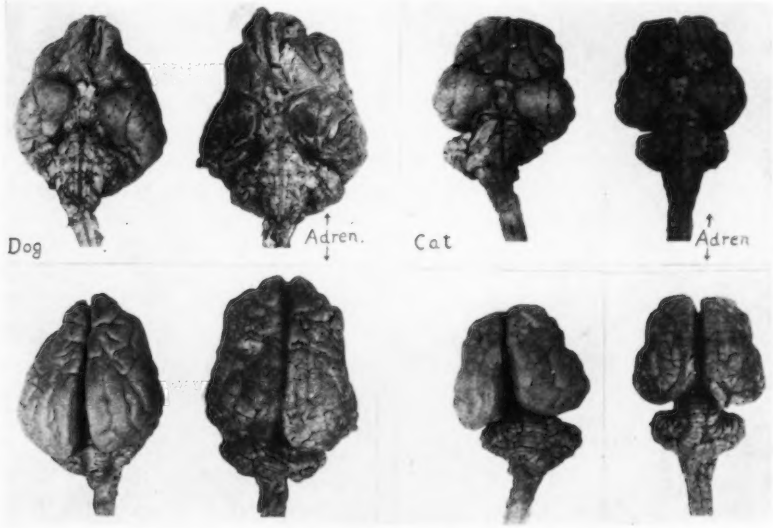

Fig. 4. Injection of cerebral circulation without and with adrenalin.

Brains of dog and cat injected with india ink through both vertebral arteries—carotids closed.

Pump output for injection in each case was the same as that used to maintain respiration earlier in experiment.

Of each pair of brains, one was injected without adrenalin, one with 1 to 3 cc. of suprarenin bitartrate (1:1,000) added to 5 cc. of the perfusing fluid (Ringer).

CO_2 inhalations were always followed by increase rather than decrease in venous outflow. Pronounced change in the vessels should have been revealed by this method; slight changes would doubtless have escaped detection.

In experiments in which the perfusion method was used, stimulation of a cervical sympathetic nerve, or addition of adrenalin to the perfusing blood, did cause rise in perfusion pressure even though pump output remained constant. But this result, when tested in detail with adrenalin,

was found to be due to constriction of extracranial vessels rather than of cerebral vessels. For when india ink or a dye such as methylene blue or trypan blue was added to the perfusion fluid it was abundantly distributed to the tongue, gums, lips, roof of mouth, and the temporal, occipital and cervical muscles in addition to the brain and upper cord unless adrenalin was also added; in which case the cerebral vessels were more completely injected while the extracranial tissues, with the occasional exception of patches in the cervical muscles, were free from dye (fig. 4).

While these results do not demonstrate the complete absence of vasomotor innervation of the brain, they add to the accumulated evidence that such innervation if it exists is of subsidiary importance. They also show that the perfusion method can be made to deal with the cerebral circulation alone, for extracranial anastomoses can be occluded effectively by means of adrenalin added to the perfusing blood, without disturbing the blood supply of the central nervous system.

c. Chemical influences. While many investigations have been made of the influence of aortic blood pressure and of nerve stimulation upon the cerebral circulation, little attention has been paid to the responses of cerebral vessels to chemical changes in the blood or tissues since the experiments of Roy and Sherrington (18), which indicated the possible importance of such responses. Their methods were criticized by Bayliss and Hill (15) who were unable to confirm their results. Tschuewsky (19) reported that the cerebral vessels dilate during acute anemia. Recently Gruber and Roberts (20) have found evidence of dilatation of perfused cerebral vessels by acid, constriction by alkali. Gesell and Bronk (21) described "circulatory adjustments in favor of the brain" during inhalation of CO_2 or during anoxemia. Tinel (22) reports that inhalation of CO_2 dilates and overventilation constricts the cerebral vessels of man. Similar change in cerebral vessels are postulated by Dautrebande and Haldane (23) in explaining the respiratory responses to oxygen inhalation, by Douglas and Haldane (24) in considering the mechanisms involved in maintaining a constant CO_2 tension within cells, and by Haldane (25) in reviewing the factors concerned in acclimatization to high altitudes. Among the factors which might influence the concentration of stimulant material within the respiratory center, changes in caliber of blood vessels supplying it must be prominent, and since it is unlikely that a competent vasomotor nervous mechanism exists in the cerebral vessels their response to chemical influences is all the more important.

The perfusion method is well suited to test the response of cerebral vessels to acute anemia, to CO_2 excess, to oxygen lack, to increased acidity or alkalinity, and to changes in temperature of the blood reaching them. Most experiments have been made with the addition of adrenalin to the perfusing blood, in order to limit the perfusion circulation to the cerebral vessels, as far as possible. The results were of the same nature with or

without adrenalin, and were constant in dogs and cats whose respiration was in good condition.

1. Acute cerebral anemia. When, after a control period, the perfusion pump is stopped for 15 to 30 seconds, and then restarted, the original height of perfusion pressure is only slowly attained (fig. 7). This can only mean that the interruption in the circulation through the perfused vessels had caused them to dilate. Dilatation could not have been caused by changes in composition of the blood due to respiratory effects of cerebral anemia; for the pump system holds enough blood to supply the cerebral circulation for ten to thirty seconds of perfusion, and hence the blood which was perfused through the cerebral vessels immediately after the interruption was blood taken from the animal's arteries *before* the cerebral anemia began. That the dilatation can be referred to cerebral vessels rather than to extracranial anastomoses is shown by its occurrence only in those cases in which the central nervous system was active. When respiration had ceased and reflexes had disappeared perfusion pressure returned immediately to its previous level when acute cerebral anemia was terminated (fig. 5 D). Evidently the dilatation of perfused vessels during acute anemia depends on products of active nerve cells. In the next paper evidence will be presented which shows that the active brain produces vasodilator substances.

2. Changes in perfusing blood. In brain perfusion experiments inhalation of oxygen by the animal was always followed by a rise in perfusion pressure: when oxygen inhalation was discontinued it fell. On the other hand, inhalation of 10 per cent CO_2 in oxygen caused perfusion pressure to fall. This result was constantly obtained when the CO_2 mixture was substituted for room air, but it was more conspicuous if pure oxygen was the gas previously inhaled. Anoxemia produced by nitrogen and followed by oxygen inhalation produced effects like those of CO_2. Examples are shown in figure 3 of the third paper of this series. In such experiments the results are not modified by the presence of adrenalin in the perfusing blood: they are therefore direct evidence of constriction of cerebral vessels by excess oxygen, dilatation by excess CO_2 or by oxygen lack.

Analogous effects are produced by changes in blood reaction resulting from addition of fixed acid or alkali. Acids dilate and alkalies constrict cerebral vessels, carbonate being much more powerful than bicarbonate (fig. 5). It is interesting to note that after cerebral activity has ceased the perfused vessels are no longer constricted by bicarbonate (fig. 5) just as they are no longer dilated by anemia (fig. 5). The effects of fixed acid and alkali have not been studied in detail, for only lactic and hydrochloric acids and sodium bicarbonate and carbonate have been used. No effort was made to keep the reaction of the perfusing blood within physiological limits.

Similarly, heat and cold affect the vessels of the perfused brain. Warming the perfusing blood to 43 to 45°C. caused a fall in perfusion pressure, cooling it to 35°C. caused a rise above the normal level. No evidence was obtained of intense cerebral vasoconstriction resulting from excessive heat, as described by Florey (26).

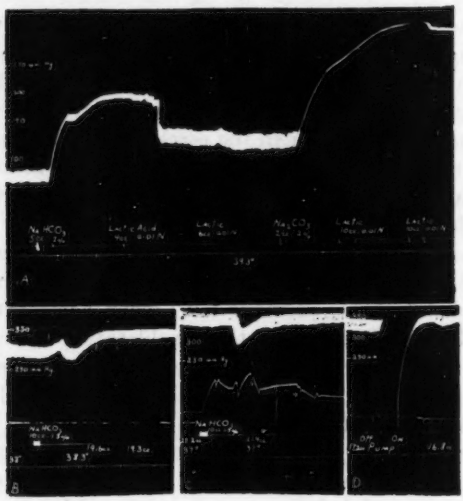

Fig. 5. Effect of alkali and acid on cerebral vessels. From two experiments on cats: vertebral perfusion.

Perfusion pressure (calibration shown) recorded by a membrane manometer, from time tracing as abscissa.

A. February 8, 1927; respiration failed about 5 minutes before this record was made; no adrenalin in perfusing blood; alkali or acid added to pump intake.

B. February 16, 1927: respiration failed less than 5 minutes before this record was made; adrenalin throughout; bicarbonate (freshly prepared and filtered) added to pump intake.

C. Same experiment as B, about 30 minutes later. The fall in perfusion pressure immediately after adding bicarbonate was due to the introduction of this watery solution into the perfusing system, for it can be duplicated with any saline solution.

D. Same experiment as B and C, about 2 minutes after C.

Note on spasm of cerebral vessels. In about half of 36 cerebral perfusion experiments on cats, it was observed that immediately after substitution of the pump perfusion for the natural circulation perfusion pressure began to rise steadily, and dyspnea appeared. In most cases the perfusion pressure fell again spontaneously and respiration became normal. But in 5 experiments the rise of pressure progressed steadily to 350 mm. Hg or more and continuation of the experiment became impossible because of cerebral anemia. The obstruction to blood-flow appeared to be due

to constriction of vessels rather than to mechanical impediment (e.g., clot) for it could be overcome temporarily by nitroglycerine or caffeine. Such spasms were encountered in perfusion experiments only, never in venous outflow experiments. Hence they may be regarded as artefacts caused by passage of the blood through the pump and possibly due to damage to formed elements in the blood. Such spasms were never produced by adrenalin or by stimulation of the cervical sympathetic and hence are not to be attributed to nervous influences.

III. THE RESPIRATORY RESPONSES TO CHANGES IN CEREBRAL BLOOD-FLOW. It has long been known that changes in cerebral blood-flow influence respiration. The extreme example is to be found in the intense stimulation of breathing,[2] followed by paralysis, which results from occlusion of the carotid and vertebral arteries (Kussmaul and Tenner (27), Rosenthal (28), Hill (29), Pike and co-workers (30), (31), Lumsden (32), Kuno (33) and many others, the literature having been reviewed by Winkin (42)). Less profound changes in cerebral blood-flow are also effective, as was shown by experiments in which the abdominal aorta was compressed and released (35), hemorrhage induced (36), (37), (31), or adrenalin injected (33), (34). These older results have been confirmed repeatedly in our experiments, and our methods have permitted more definite conclusions. For the venous outflow method furnishes an unmistakable index of the direction of a change in cerebral blood-flow, as well as an approximation of its extent, while the perfusion method is particularly well adapted to the study of this question because, within certain limits, it permits the elimination of such important variables as changes in the gaseous composition of the blood and simultaneous changes in blood-flow through tissues other than the brain.

Acute anemia of the brain, produced by clamping the carotid and vertebral arteries, produces dyspnea (fig. 6). In the cat (fig. 6 A) dyspnea begins immediately and progresses for about 20 seconds as a rule, after which terminal gasps occur and respiration practically always fails within 30 seconds of occlusion; if the clamps are removed during the dyspnea apnea results; if after respiratory failure, respiration returns. In the dog (fig. 6 B) the respiratory responses are not so abrupt, probably because of the greater importance of the anterior spinal artery, but they are of the same quality.

These effects might be explained by changes in the gaseous content of

[2] An outstanding exception to this conclusion is taken by Roberts (34) who regards immediate failure as the typical respiratory response to acute cerebral anemia. We have had occasion to make the experiment on more than 100 cats and on some 50 dogs, and have never failed to find hyperpnea before respiration failed, unless the animals were very deeply narcotized or the blood pressure was very low for some time before cerebral anemia was produced.

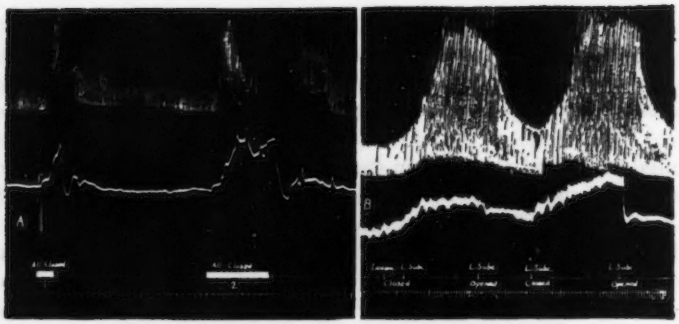

Fig. 6. Respiratory and circulatory responses to acute cerebral anemia. A—cat; B—dog.

Records, from above down: Respiratory volume: femoral arterial pressure, to be measured from time tracing; signal; time in 5 seconds.

All tracings to be read from left to right.

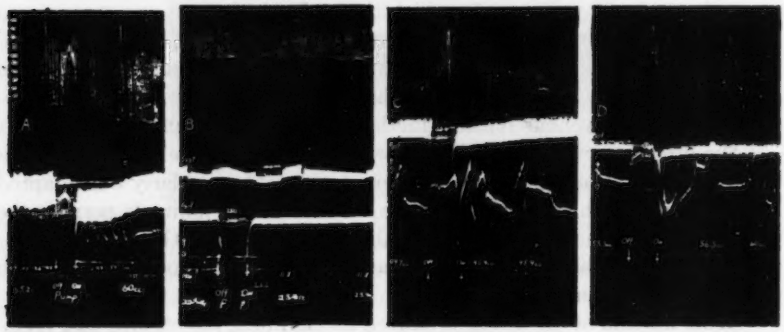

Fig. 7. Acute cerebral anemia in perfusion preparations. A—dog; B, C, and D—cats.

Pump perfusion of both vertebral arteries, common carotids and all subclavian branches closed except vertebrals.

In A, perfusion and systemic pressure were both recorded by mercury manometers, the time tracing being the zero level. In all the cat experiments, perfusion pressure was recorded by a Huerthle membrane manometer, the zero level of which is the line next below the perfusion pressure tracing. In B, systemic pressure was also recorded by a membrane manometer, but in C and D, a mercury manometer was used, zero level being the time tracing.

The transverse lines near the perfusion pressure tracing show calibration of the manometer used, in millimeters of mercury.

The respiratory volume (upper) record shows a calibration in cc. in A; the same calibration applies to the other records. Time record shows 5 second intervals.

In each case, the pump was restarted before respiration began to fail; the respiratory muscles relaxed immediately and apnea occurred in each case.

the blood—that readmitted during dyspnea being relatively poor in CO_2, that readmitted after respiratory failure being rich in CO_2 and poor in oxygen. That this is not the real explanation can be shown by performing the experiment while the animal is breathing CO_2 or nitrogen (fig. 8). The existing dyspnea is intensified by clamping the cerebral vessels, but apnea occurs when they are reopened. In this case the apnea could scarcely be ascribed to absence of stimulant material in the blood. It must be due to restoration of blood-flow through the brain. Further evidence that this is the chief cause of the apnea is found in experiments with the perfusion method (fig. 7). When the pump is stopped dyspnea

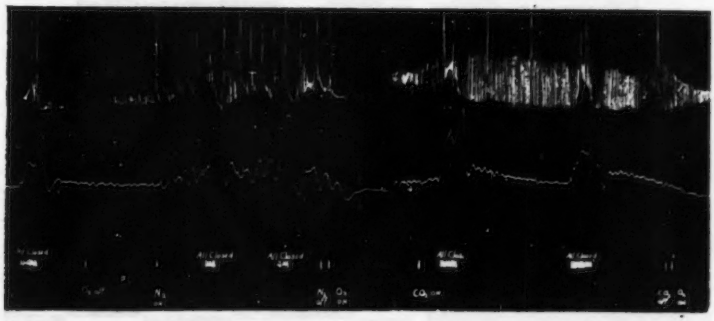

Fig. 8. Respiratory depression resulting from release of occluded cerebral vessels during inhalation of CO_2 or nitrogen.

Cat: A and B from one experiment.

CO_2 (10 per cent in oxygen) or nitrogen inhalation through a cup held over the tracheal cannula.

Occlusion of common carotid and vertebral arteries ("all closed") increased the dyspnea in each case, and vessels were released before this added dyspnea decreased. In each case, release of the vessels caused immediate relaxation of respiratory muscles and a sharp fall in blood pressure; the coincidence of these events is not shown accurately on the tracings, for the blood pressure record was about 2 mm. ahead of the respiratory record.

begins at once; on starting it again apnea occurs. In these cases, because of the capacity of the pump system, we know that the blood responsible for the apnea was taken from the animal before the dyspnea began—that is, before cerebral blood-flow was interrupted.

Respiratory responses to changes in systemic blood pressure were routinely observed during the course of the experiments mentioned above (Section II A), in which cerebral blood-flow was found to vary directly with systemic pressure. Respiration was found uniformly to vary inversely with cerebral blood-flow, within certain limits. Examples are shown in figure 2 of the third paper of this series. In cerebral perfusion experiments also respiration is immediately stimulated by a decrease in

pump output, immediately depressed by an increase (fig. 9), but respiration tends to return to normal though the change in perfusion flow continues.

Before the respiratory responses in these experiments can be attributed wholly or largely to changes in cerebral blood-flow account must be taken of two other possibilities. One of these concerns changes in intracranial

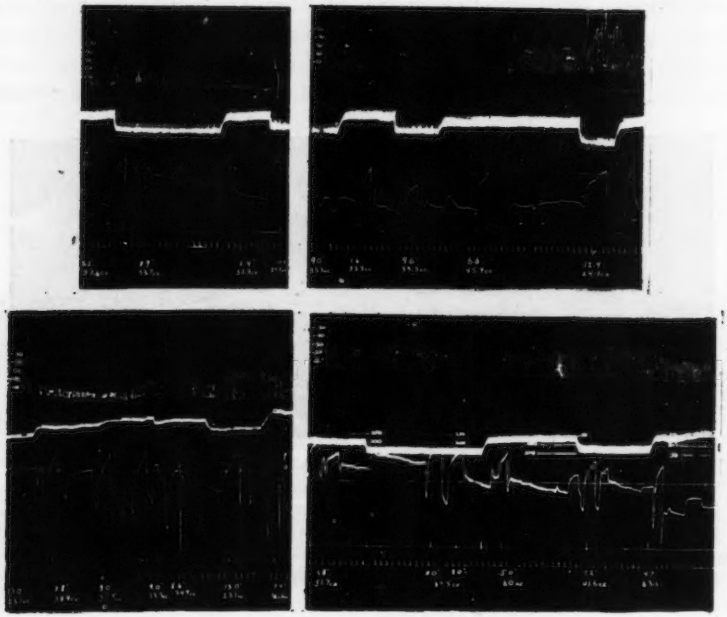

Fig. 9. Respiratory responses to changes in cerebral perfusion flow.

Pump perfusion of both vertebral arteries, carotids closed. Four different experiments on cats.

Records, from above down: Respiratory volume; perfusion pressure (Huerthle manometer); zero for perfusion pressure; systemic blood pressure (mercury manometer); signal; time in 5 seconds—also zero for systemic pressure.

Numbers show time (seconds) for 5 cc. of blood to be delivered by pump; the volume of output (cubic centimeters per minute) is also shown.

tension. Although known effects of changes in intracranial pressure are not such as to lead one to think that they could cause such respiratory responses, experiments were made on 10 cats in which the cranio-vertebral cavity was opened either by trephining the skull or dividing the occipito-atlantoid ligament or both. The results were similar in nature and degree to those in animals whose cranio-vertebral cases were intact.

The second possibility is that of reflex inhibition of respiration resulting

from a rise in aortic pressure, reflex stimulation from a fall. The work of Heymans and Heymans (38) indicates that such reflexes may take part in the production of apnea by adrenalin and other pressor agents, and in the production of hyperpnea resulting from depressor procedures. That such reflexes are not the sole cause of respiratory responses to changes in cerebral blood-flow is shown by the results of cerebral perfusion experiments, in which cerebral flow was altered while systemic pressure was not. Also, these reflexes are carried by the vagus nerves (38), yet it is possible to produce respiratory depression or apnea in vagotomized animals by intravenous injection of adrenalin (39)—an observation which we have confirmed, not only for adrenalin, but also for infusion of blood or gum saline. In certain perfusion experiments we have found that a rise in aortic pressure had little effect on respiration until cerebral blood-flow was allowed to increase, when apnea occurred at once. An example is shown in figure 10. Further evidence that an abrupt increase in cerebral blood-flow can produce apnea apart from aortic reflexes is found in the effects of occlusion and release of cerebral arteries: here dyspnea is associated with a rise in aortic pressure, apnea with an abrupt fall.

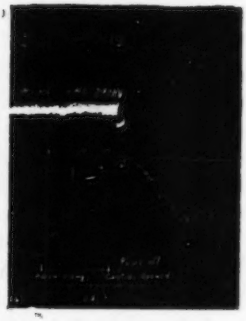

Fig. 10. Effect of adrenalin on respiration in perfusion preparations.

Cat: Perfusion of both vertebral arteries, carotids closed. Adrenalin, added to pump intake, had little effect on perfusion pressure or respiration, though systemic pressure rose markedly; on opening carotids apnea occurred at once, and persisted after the perfusion pump was stopped.

We conclude that the respiratory center is influenced directly by changes in its blood supply, and that the respiratory responses to changes in systemic blood pressure depend more upon changes in cerebral blood-flow than upon reflexes aroused by changes in aortic pressure. But such reflexes are undoubtedly a factor in the respiratory responses: this is evident in the fact that a given rise in blood pressure produces less respiratory depression after vagotomy than before. It is practically always possible to produce apnea by raising blood pressure in the vagotomized animal if the rise be great enough, but the pressure level required is well above that which produced apnea before the vagi were cut.

IV. The reversal. In the preceding pages attention has been directed to the inverse relation between cerebral blood-flow and respiratory activity. This relation suggests that products of the metabolism of the cells of the respiratory center play a part in regulating the activity of the center and in the following paper we shall show that these cells possess an active metabolism which is increased by agencies which stimulate respiration.

We wish now to consider an abnormal condition of the center in which respiration is depressed by a decrease in its blood supply, stimulated by an increase. An example is the respiratory response to acute cerebral anemia: if the anemia is terminated during the dyspneic period, apnea occurs, but if it be continued until respiration fails, restoration of cerebral blood-flow causes a return of breathing. Under conditions to be mentioned below other respiratory stimulant agencies may produce depression of breathing. For the sake of brevity we shall designate this abnormal relation, which is the reverse of the normal, as the Reversal.

In trying to analyze the conditions required for the production of the Reversal we find, first, that it is never produced by excess CO_2 in the blood, so long as blood oxygen content is not markedly reduced and the cerebral circulation is adequately maintained; second, that it is readily produced by general anoxemia or by reduction in cerebral blood-flow; third, that any agent, normally stimulant to respiration, may cause depression or failure if applied in the presence of oxygen-lack in the brain consequent upon subnormal cerebral blood-flow.

Thus (fig. 11 A) inhalation of CO_2 in concentration as high as 80 per cent has never been anything but stimulant to breathing in these experiments, provided that the inspired air contained 20 per cent or more of oxygen and the cerebral circulation was adequately maintained during the inhalation. But acute general anoxemia (fig. 11 B) carried to the limit of tolerance was not only less stimulant to breathing than strong CO_2, but was likely to lead to respiratory depression. Evidently the ability of the center to respond to stimulant material carried to it by the blood depends upon an adequate supply of oxygen.

We conceive the products of metabolism of cells of the respiratory center to be the same as those of other body cells. The center is therefore stimulated by reduction in its blood supply just as it would be by increase in the CO_2 or acid of the blood. But if reduction in blood oxygen depresses the center, similar depression should be produced by oxygen-lack limited to the brain. The phenomena of acute cerebral anemia show that this is true of the cat (fig. 6 A), and of the dog also (fig. 12). In the latter, it is usually necessary to resort to repeated intermittent occlusions of the cerebral arteries, for one continued occlusion seldom produces continued stimulation, and almost never causes the Reversal. The subacute cerebral anemia produced by such intermittent occlusion in the dog (fig. 12) causes a progressive delay in recovery from the occlusions until finally dyspnea persists; soon afterward respiration fails, but it can be restored by improving cerebral blood-flow. The progressive fall in blood pressure which coincides with the development of the Reversal suggests that the vasomotor center is also undergoing depression.

Evidently products of the activity of the cells of the center, accumu-

lated there because of reduction in its blood supply, are first stimulant, finally depressant, to its functional activity. Since an accumulation of CO_2 alone is purely stimulant (fig. 11) while measures which reduce in-

Fig. 11. Inhalation of strong CO_2 and of nitrogen—cat.

Respiratory volume and blood pressure records only—cat. Barbital narcosis.

CO_2, 75 per cent; O_2, 25 per cent, inhaled from large spirometer, through cup fitting loosely about tracheal cannula—gas supplied under pressure of about 2 mm. Hg.

Nitrogen inhaled in same way, using somewhat higher pressure.

Tracing B was made 3 minutes after tracing A.

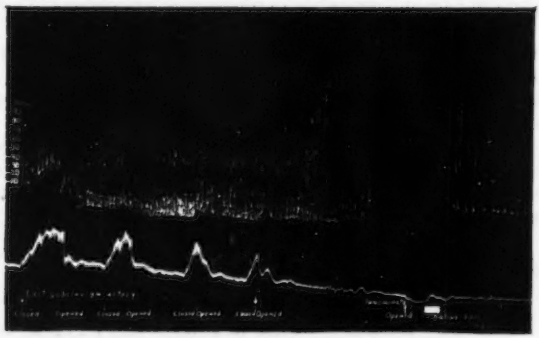

Fig. 12. Effect of continued subacute cerebral anemia—dog.

Barbital narcosis. This tracing was made later in the experiment from which figure 6 B was obtained. Innominate artery closed throughout to indicated point of release.

tracellular oxygen are able to cause depression, it is evident that the ability of the respiratory center to respond to stimuli depends upon the presence of a supply of oxygen adequate to support the metabolic processes upon

which the functional activity of its cells depends. Products of metabolism of other tissues, or of the center itself, are stimulant to breathing as long as the existing supply of oxygen is sufficient for the needs of the cells of the center: they are depressant when the oxygen supply is insufficient. A sustained reduction in cerebral blood-flow may not prevent the respiratory response to CO_2 inhalation, but it minimizes or prevents a stimulant response to general anoxemia (fig. 13), which produces prompt respiratory failure in the presence of subacute cerebral anemia. The Reversal, therefore, appears to be due to a derangement of the vegetative functions

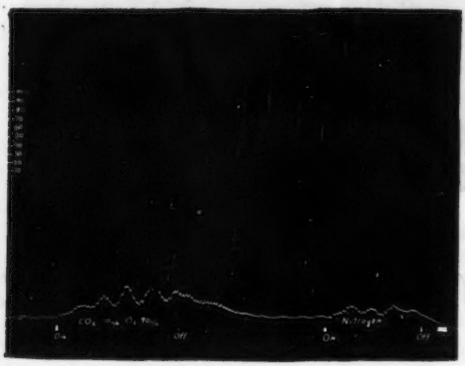

Fig. 13

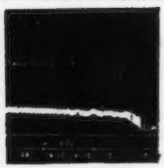

Fig. 14

Fig. 13. Effect of CO_2 and anoxemia in presence of prolonged circulatory depression—cat.

From same experiment as figure 11—follows repeated small bleedings to lower blood pressure. CO_2—10 per cent in oxygen—inhaled as above.

Fig. 14. Respiratory failure following CO_2 inhalation—dog. Urethane narcosis. Morphine 6 mgm. per kilo by vein. Respiration had been maintained as shown for 30 minutes. CO_2 6 per cent in air—inhaled for one minute—this had produced only respiratory stimulation before. Infusion of saline with adrenalin subsequently restored circulation and respiration. Time tracing is abscissa for blood-pressure record. Numbers indicate rate of respiration per minute.

of the cells of the center rather than to a "narcotic" action exerted by excess CO_2 (40).

It follows that any measure which increases the oxygen consumption of the center will cause respiratory depression or failure, if the oxygen supply is barely adequate for the basal requirement of its cells. We shall show in the next paper that respiratory stimulants increase the oxygen consumption of the brain. Any effective stimulant might therefore cause the Reversal if applied in the presence of reduction in cerebral blood-flow. This is true of CO_2 inhalation (fig. 14) and of respiratory stimulant drugs such as atropine, camphor, strychnine and caffeine (fig. 15). The Re-

versal following CO_2 inhalation is not common, but its occasional occurrence is noteworthy. Of the drugs, atropine is most likely to cause the Reversal, caffeine least, but while atropine may properly be regarded as depressant as well as stimulant to the central nervous system, caffeine is ordinarily held to be purely stimulant (41). In any case, it should be noted that the Reversal never follows CO_2 or caffeine if the circulation is improved before they are applied, and that circulatory stimulants are usually able to overcome the respiratory depression produced by them (fig. 15).

We therefore regard as valid the assumption that an effective respiratory stimulant may lead to exhaustion of the oxygen within the cells of the respiratory center, thus producing the Reversal, if it is applied when cerebral blood-flow is subnormal. As definite evidence that it is the

Fig. 15. Respiratory depression produced by caffeine, recovery following a circulatory stimulant (ephedrin).

Cat—urethane narcosis; follows a total of 200 mgm. of morphine sulphate by vein. Respiration had been perfectly adquate and steady for 25 minutes before caffeine was given. Note the gradually increasing improvement of circulation and respiration produced by the ephedrine.

stimulation which precipitates respiratory failure, we present the results of experiments in which the Reversal was induced by direct electrical excitation of the medulla in the presence of reduced cerebral blood-flow. Such an experiment is shown in table 1. Either acute cerebral anemia or electrical stimulation stimulated breathing. Cerebral (perfusion) flow was then decreased to a level which, while subnormal, was evidently adequate for the maintenance of respiration. The same electrical stimulus now caused depression of breathing. The data concerning the behavior of cerebral gaseous metabolism in this experiment are given in the next paper (table 5), in which evidence is presented that the Reversal is accompanied by a prolonged diminution in the ability of the brain cells to utilize oxygen.

The Reversal therefore appears to be due to accumulation of products of incomplete oxidation within the cells of the respiratory center. It can be induced by general anoxemia or by reduced cerebral blood-flow, alone or combined, as well as by stimulation of the center in the presence of a reduced blood supply. The respiratory responses to changes in cerebral blood-flow are the reverse of normal because in this case the change in blood-flow entails a change in concentration of depressant instead of

TABLE 1

Production of the reversal by electrical stimulation of the medulla in the presence of reduced cerebral blood-flow

July 15, 1926. Vertebral perfusion experiment: Dog, 7.8 kgm.: barbital narcosis. Adrenalin in perfusing blood throughout.

Medulla stimulated by unipolar electrode inserted through hole drilled in atlas; other terminal attached to left hind leg through copper electrode and cotton soaked in saturated NaCl solution. Induction current of same strength was used in both cases.

Respiration could not be restored by subsequent increase in pump output.

TIME	CEREBRAL FLOW (PUMP)	RESPIRATION			CONDITIONS
		Rate	Depth	M-V	
	cc. per min.				
2:47	54	16	60	960	Control—adequate flow
2:59	0	28	70	1960	Pump stopped at 2:58, started at 2:59:55
3:00	57	0	0	0	Immediately after restarting pump
3:09	56	20	65	1300	Recovery—adequate flow
3:16	57	28	45	1160	Same
3:17	57	40	60	2400	Electrical stimulation of medulla—adequate flow
3:20	57	24	50	1200	Stimulation off at 3:18
3:27	24	20	85	1700	Pump slowed at 3:20—subacute anemia
3:34	24	40	30	1200	Subacute anemia continuing
3:36	24	10	35	350	Electrical stimulation of medulla—reduced flow. Stimulation off at 3:34:40
3:39	24	6	10	50	Weak gasps continued until 3:42

stimulant material within the center. The nature of this depressant material has not been determined.

SUMMARY

1. Methods are described for measuring cerebral blood-flow as arterial inflow and venous outflow, and for perfusing the brain with a measurable volume of blood, in spontaneously breathing cats and dogs.

2. Evidence is presented that cerebral blood-flow varies with changes in systemic blood pressure and is not subject to direct vasomotor influences

of sufficient strength to be detected by these methods. The cerebral vessels are shown to be under intrinsic chemical control, for they are dilated by CO_2, anoxemia, acid, heat and cerebral anemia, constricted by excess oxygen and alkali, cold, and probably by increased blood-flow.

3. Respiration is depressed by an increase in cerebral blood-flow, stimulated by decrease, no matter how produced, though within certain limits only. Respiratory depression follows a marked increase in cerebral flow even during CO_2 or nitrogen inhalation. Similar results are obtained when the vagi are cut, and whether systemic blood pressure rises or falls, showing that reflexes from the heart and aorta are not the sole cause of the respiratory response; it is also shown that a rise in blood pressure may have little or no effect on respiration until cerebral blood-flow is allowed to increase. The respiratory responses are not due to changes in intracranial tension.

4. Beyond a certain limit a reduction in cerebral blood-flow depresses breathing and an increase then stimulates respiration; to this condition the term Reversal is applied. It is shown to be due to an insufficient supply of oxygen to the brain, and can be produced by decreased blood oxygen content, by decreased cerebral blood-flow, or by stimulation (electrical, by drugs or by CO_2 inhalation) in the presence of reduced cerebral blood-flow. It is prevented or removed only by an improvement in cerebral oxygen supply, most effectively through an improvement in cerebral blood-flow.

5. These results explain the paradoxical relations between blood pressure and respiration which led to these experiments, and are presented as additional support to the theory of Gesell.

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THE INFLUENCE OF CEREBRAL BLOOD-FLOW ON RESPIRATION

II. The Gaseous Metabolism of the Brain

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One of the propositions made at the beginning of this study was that respiration varies with the degree of accumulation of stimulant material within the cells of the respiratory center. The results described in the preceding paper, which show that the center is usually stimulated by a decrease and depressed by an increase in cerebral blood-flow, support this proposition. It is now necessary to inquire whether the metabolism of the central nervous system is of sufficient intensity to justify the belief that the concentration of CO_2, oxygen, or acid within the center is affected by alterations in cerebral blood-flow. For obvious reasons it is impossible to estimate the metabolism of the respiratory center as a separate structure. Our efforts have been devoted to comparison of the gas contents of blood flowing into and out of the brain under different conditions of cerebral activity as measured primarily by the state of respiration. If it can be shown that the gas metabolism of the brain increases during hyperpnea and decreases during apnea we may regard this as evidence that the metabolism of the cells of the respiratory center can contribute to the conconcentration of stimulant material within them.

Existing evidence concerning cerebral metabolism does not answer our purpose. Hill and Nabarro (1) measured the oxygen and CO_2 content of blood collected from the torcular Herophili before and during convulsions caused by absinthe. Their conclusion—that the metabolism of the brain is much less than that of the leg muscles, both at rest and during convulsions—is based on the assumption that the volume of blood-flow through the two structures was of the same order at the start, and was altered to the same degree in both by the convulsions. This assumption is open to serious question.

Alexander and his associates (2), (3), (4) concluded that the oxygen consumption of the brain was augmented by stimulation of the retina with bright light, and that both oxygen consumption and CO_2 production were

depressed by ether, morphine, and magnesium salts. The factor for cerebral blood-flow used in their calculations was obtained by measuring the rate of outflow of blood from a cannula in the sagittal sinus. Such a factor is valueless unless account is taken of the pressure existing in the superior cava, and there is nothing to indicate that this was done. Also the very low concentration of oxygen in cerebral venous blood in their experiments arouses suspicion of stasis in the collecting system.

The evidence in the results of other investigators regarding the gaseous metabolism of the brain and its behavior under conditions of altered cerebral activity is therefore scarcely convincing.

Several attempts have been made to estimate the intensity of cerebral metabolism from the standpoint of carbohydrate utilization (5), (6), (7), (8), (9), (10) and of fixed acid production in the excised brain (11), (12). The results are of interest in that they point to active utilization of carbohydrate in the brain and a marked augmentation in this utilization following convulsant drugs, while lactic acid has been found to accumulate in the freshly excised brain (12). Of particular importance is the demonstration (12) that prolonged anoxemia (carbon monoxide inhalation) apparently leads to accumulation of lactic acid in the brain. Such results are very suggestive, but it is difficult to apply them directly to the question of respiratory control since they were obtained through analyses of excised brain tissue: the relation of the change in carbohydrate or lactic acid content to changes within the respiratory center under physiological conditions is wholly a matter of inference, and there is no means of knowing whether the changes indicated by the analyses are expressions of the stimulant or depressant (reversal) type of effect upon the center.

It appeared to us to be of utmost importance that natural respiration be present while cerebral metabolism was being estimated, if we expected to obtain results that could be applied to the regulation of breathing. The best criterion of cerebral metabolism available under these conditions seemed to be found in estimations of the oxygen consumption and CO_2 production of the brain, particularly of the brain stem or medulla. The chief difficulty in such determinations on any organ lies in the technical problems (preparation of an isolated circulation, collection of arterial and venous blood without interference with the organ's circulation, and above all accurate estimation of the volume of blood-flow corresponding with the blood samples). In the case of the brain these physiological difficulties are in some respects greater, because of the impossibility of obtaining an isolated circulation either on the arterial or venous side, but with proper procedures it is possible so to control the variables introduced by this anatomical peculiarity that the gaseous metabolism of the brain can be estimated with sufficient accuracy to justify conclusions. These procedures will be described below.

METHODS. The method of perfusion of the vertebral arteries has been used in obtaining the results to be described. Its advantages for the purpose are these: First it permits the maintenance of a constant cerebral blood-flow in spite of changes in systemic pressure which may occur spontaneously, and which inevitably follow the withdrawal of blood samples for analysis. Second, it furnishes a means of measuring cerebral blood-flow with reasonable accuracy, for while an unmeasurable part of the perfused blood may escape from the Circle of Willis to extracranial vessels, this escape can be decreased to a negligible degree by means of the addition of adrenalin to the perfused blood; complete accuracy cannot be secured because of the communication between anterior spinal and basilar arteries, but if the perfusion pressure be maintained at a higher level than systemic this vessel cannot add to cerebral blood-flow; on the other hand, it is inevitable that some of the perfused blood may reach the anterior spinal distribution under these circumstances, but the adrenalin will prevent its escape from the vessels of the spinal cord, so that this source of error is inconsiderable. Third, it deals wholly with the arterial circulation, so that venous return from the brain is not affected. The perfusion preparation, with adrenalin added to the perfusing blood and with the pump set at an output which maintains perfusion pressure above systemic, is therefore the method of choice. The collection of venous blood which shall reveal the gaseous metabolism of the brain is of outstanding importance. In the preceding paper it has been stated that the right vertebral vein of the dog (either vertebral vein of the cat) is the most direct channel for the return of blood from the medulla.

Collection of blood from the right vertebral vein is difficult in breathing dogs because it enters the cava a centimeter or more central to the union of the innominates. We have accomplished it by means of a small glass tube, suitably curved, the tip of which was introduced into the cava through an internal mammary vein, and manipulated so that it passed the valve at the vertebral orifice and entered the right vertebral vein. The tube was closed by a rubber connection and the vertebral vein was tied tightly upon the part of the tube within its lumen, so as to prevent reflux of blood from the cava. This of course obstructed outflow through the right vertebral vein but no symptoms ever resulted, the other pathways being perfectly adequate. Before collecting blood from this vein the tube was opened and blood allowed to escape for 2 to 4 minutes, to allow for the stasis produced by closing the vein.

Another method depends upon the torcular Herophili which is more accessible and the preparation is much easier. It involves simply exposure of the occipital protuberance, drilling a hole into its most prominent part until blood escapes freely, and inserting a suitable metal cannula which must of course be closed by a rubber connection. This taps a venous

reservoir but does not interfere with return of blood from the brain. Before collecting samples for analysis blood was allowed to ecape from the cannula for a minute or two, to eliminate possibly stagnant blood from the sample.

In 4 experiments we collected blood simultaneously from right vertebral vein and torcular (dogs). These samples were similar but not identical as to gas content, that from the vertebral being constantly a little more venous, and when the medulla was stimulated or depressed both samples showed a change in the same direction, but it was rather more marked in the vertebral sample. If it were possible to be certain of the source of blood collected from these veins such results would point to a higher grade of metabolism in the medulla than in the higher parts of the brain, but in view of the free communications between vertebral and torcular systems this conclusion is scarcely justified. For this reason the results of this rather laborious effort are not presented in detail, and we conclude that blood samples collected by the simple torcular method are capable of answering the same questions as are those obtained by the difficult vertebral method. In cats it is very difficult to collect vertebral venous blood, and the torcular method is the only one worth considering.

We have taken pains to confirm the source of our cerebral venous blood samples by injection of dyes after every experiment into the venous cannula. These have shown—in every experiment from which data are presented or conclusions drawn—no staining of veins other than cervical spinal, medullary, and cerebral sinuses, unless excessive pressure was used: in this case the injected fluid reached the azygos system, and thence the cava, heart, and pulmonary arteries. In some cases parietal emissary veins were stained.

The technical procedures involved in collection and analysis of the blood samples were as follows: Collection was made into 5 cc. glass syringes, using a minimum of suction, and arterial and venous samples were drawn synchronously, 0.5 cc. for 0.5 cc. The samples were transferred to pyrex tubes under oil and a few crystals of potassium oxalate were added as an extra precaution against clotting. Analyses were begun as soon as possible and were completed within 8 hours after collection. The method used was that of Van Slyke and Neill (13), using 1 cc. samples of blood, upon which oxygen and CO_2 contents were determined. Duplicate analyses were made routinely at first until we were able to obtain results that agreed consistently within 0.2 volume per cent. In the later experiments duplicate analyses were made on a few of the samples, and were always made in cases of unusual results. In some experiments lactic acid content of arterial and cerebral venous blood was determined by the method of Clausen (14); these were always made in duplicate, but the results were so inconsistent that they deserve no consideration. We hoped to estimate

pH of the blood samples, but were unable to obtain useful results: Cullen's method (16) failed because some hemolysis was usually present; the dialysis method of Dale and Evans (15) showed frequent wide variations which we could account for only by assuming a combination of the anesthetic (barbital) with the indicator; the quinhydrone electrode yielded erratic results; there was not enough blood for estimation by means of the hydrogen electrode.

In almost all experiments a current of oxygen was maintained throughout by means of a tracheal catheter. Having obtained blood samples during a control period of quiet breathing, respiratory activity was altered by stimulation of a central vagus; by direct electrical stimulation or by puncture of the medulla; by inhalation of CO_2 (10 per cent in 90 per cent oxygen) or by rebreathing; and by drugs, such as morphine, atropine, caffeine, ether, and cyanide. All the experiments to be reported were made on dogs.

RESULTS. *1. The gaseous metabolism of the brain during quiet breathing.* The results of nine experiments on dogs are shown in table 1. The variations in apparent oxygen consumption and CO_2 production by the brain are quite wide, but if one compares the degree of this apparent metabolism with the state of respiratory activity there is evident a tendency of the two to vary together.

In all the experiments shown in table 1 adrenalin was added to the perfusing blood. It will be noted that in experiments 1 and 2 the addition of adrenalin caused a decrease in apparent metabolism of the brain. This is to be explained by increased volume of blood perfused through the brain despite constancy of output of the pump, consequent upon constriction of vessels in extracranial tissues.[1] The figures obtained with adrenalin perfusion more truly represent actual cerebral metabolism, and in the other seven experiments only results so obtained are given.

2. The effect of changes in respiratory activity. Because of the complexity of the cerebral circulation and the difficulty in duplicating experimental conditions dealing with it, one is scarcely justified in comparing the metabolism results of one experiment with those of another, and in drawing any conclusions from such comparison. Therefore, while the results shown in table 1 suggest that cerebral metabolism varies with respiratory activity, it is only from experiments in which the apparent cerebral metabolism of the same animal is shown to vary with changes in respiration, all else being kept as nearly constant as possible, that definite conclusions may be derived. Such results are presented in tables 2, 3 and 4. All these experiments were made on dogs.

[1] It is commonly stated (17) that adrenalin has a direct depressant action on the respiratory center. That no such depression occurs when an increase in cerebral blood-flow is prevented can be shown regularly, and experiments demonstrating this fact are to be reported elsewhere.

TABLE 1

NUMBER		BRAIN WEIGHT	PUMP OUTPUT	CEREBRAL METABOLISM*		OXYGEN CONTENT		CO_2 CONTENT		RESPIRATION			CONDITIONS
				O_2	CO_2	Artery	Vein	Artery	Vein	Rate	Depth	M-V	
		gms.	*cc./ min.*	*cc. per gram per min.*	*cc. per gram per min.*								
1	A	51.7	71.43	0.1483	0.1594	15.07	4.33	10.41	21.94	36	55	1980	Before adrenalin. Time 0
	B		69.76	0.1084	0.0964	15.78	7.74	9.94	17.09	28	60	1680	Adrenalin 0.27 mgm. per minute. Time 11 minutes
2	A	59.3	53.57	0.0815	0.0634	16.05	7.02	32.37	39.38	48	50	2400	Before adrenalin. Time 0
	B		53.57	0.0669	0.0501	15.08	7.66	33.72	39.27	20	60	1200	Adrenalin 0.13 mgm. per minute. Time 13 minutes
3		53.2	88.23	0.0428	0.0688	6.40	3.82	13.14	17.29	10	15	150	Very anemic
4		53.2	61.22	0.0488	0.0559	20.63	16.39	28.48	33.34			165	Condition very poor
5		59.3	49.18	0.0519	0.0658	23.10	16.82	13.89	21.82	20	32	640	Condition fair
6		35.0	43.16	0.0693	0.1012	18.57	12.94	16.88	25.08	18	40	740	Condition good
7		65.4	60.00	0.0500	0.0523	13.97	8.52	33.40	39.10	20	45	900	Condition good
8		66.1	56.60	0.0897	0.0847	16.35	5.86	19.17	29.07	40	45	1800	Condition very good
9		42.6	58.82	0.1492	0.1408	23.38	10.49	22.80	33.36	50	42	2100	Respiration and reflexes very active
				0.0752	0.0797							1050	Average—with adrenalin

* Presentation of these data in terms of gas metabolism per gram of tissue per minute implies a quantitative significance that is not justified, for two reasons. First, the weight of tissue concerned in the metabolism could not be determined accurately: we arbitrarily used the weight above the lower end of the medulla, though terminal injections showed that the perfused dye had reached the spinal vessels, sometimes as far down as the lower dorsal region; we think it improbable that the perfused blood had been similarly distributed while the heart was maintaining a spinal circulation, but cannot state the degree of difference. Second, the figure for volume flow of blood (pump output) was artificially set at a level that may or may not have been close to the animal's normal cerebral blood-flow. We chose the perfusion method to permit comparison of our results among themselves, but these figures for gas metabolism are not to be compared with those obtained in organs in which an isolated circulation can be obtained under more nearly normal conditions.

A. *Physical agents.* The effects on apparent cerebral metabolism of direct electrical stimulation of the medulla, of strong stimulation of a central vagus and of puncture of the medulla, are shown in table 2. The

change in apparent cerebral oxygen consumption, while not very striking, is uniformly in the same direction as the change in respiration.

TABLE 2

Changes in apparent cerebral metabolism during physical changes in activity of respiration

NUMBER		CEREBRAL METABOLISM		RESPIRATION			CONDITIONS
		O_2	CO_2	Rate	Depth	M-V	
		cc. per gram per min.	*cc. per gram per min.*				
1	A	0.0975	0.1068	48	20	960	Quiet breathing
	B	0.0686	0.0292	0	0	0	Central vagus stimulation
	C	0.1017	0.1130	32	65	2080	Electrical stimulation of medulla
2	A	0.0361	0.0036	16	5	80	Respiration near failure
	B	0.0396	0.0087	20	10	200	Electrical stimuli of medulla
	C	0.0232	−0.0219	0	0	0	Medulla punctured
3	A	0.0472	0.0433	20	130	2600	Quiet breathing
	B	0.0383	0.0017	0	0	0	Medulla punctured
4	A	0.0660	0.0614	24	60	1400	Quiet breathing
	B	0.0878	0.0536	40	25	1000	Electrical stimulation of medulla
5	A	0.0922	0.0885	40	15	600	Quiet breathing
	8	0.0885	0.0446	12	40	480	Central vagus stimulation
6	A	0.1293	0.1227	40	90	3600	Quiet breathing
	B	0.0981	0.1038	8	150	1200	Respiration failing spontaneously
	C	0.0955	0.0906		Spasm		Strong electrical stimulation of medulla

Expt. 1.* June 28, 1926. Brain weight 79.8 grams. Oxygen in trachea, no adrenalin. Perfusion begun at 3:42:10. Vertebral samples.

A. Time 4:10. Pump 75 cc. per minute. Blood analyses: O_2(A)14.83 − (V)4.46 = 10.37 per cent or 7.778 cc. per minute. CO_2(V)33.63 − (A)22.25 = 11.38 per cent or 8.535 cc. per minute.

B. 4:20. Central end of left cut vagus stimulated—no respiratory movements during collection of blood. Pump 77 cc. per minute. O_2(A)14.98 − (V)7.20 = = 7.78 per cent or 5.991 cc. per minute. CO_2(V)34.96 − (A)31.92 = 3.04 per cent or 2.231 cc. per minute.

C. 4:35. Medulla stimulated by unipolar electrode. Pump 75 cc. per minute. O_2(A)14.40 − (V)3.57 = 10.83 per cent or 8.123 cc. per minute. CO_2(V)36.59 − (A)24.56 = 12.03 per cent or 9.023 cc. per minute.

* Selected protocols are presented because of space limitations.

B. *CO_2 inhalation.* The results, in four animals, are shown in table 3, together with the comparable results of a single rebreathing experiment.

These results were unexpected, for in 3 of the 4 cases apparent cerebral oxygen consumption was decreased during CO_2 inhalation. In the light of results obtained with other measures designed to alter respiration, it is evident that CO_2 is the only outstanding exception among respiratory stimulants to the rule that apparent cerebral oxygen metabolism is increased by them.

TABLE 3

Effect of CO_2 inhalation on apparent cerebral metabolism

NUMBER		CEREBRAL METABOLISM		RESPIRATION			CONDITIONS
		O_2	CO_2	Rate	Depth	M-V	
		cc. per gram per min.	*cc. per gram per min.*				
1	A	0.0947	0.1158	50	30	1500	Quiet breathing
	B	0.0759	0.0397	56	70	3920	CO_2 inhalation
	C	0.0747	0.1315	52	40	2080	Recovery
	D	0.0933	0.968	52	60	3120	Rebreathing
2	A	0.0850	0.0981	40	80	3200	Quiet breathing
	B	0.0941	0.0652	50	100	5000	CO_2 inhalation
	C	0.0788	0.1017	60	40	2400	Recovery
3	A	0.0427	0.0688	10	55	520	Quiet breathing
	B	0.0388	−0.0135	13	70	910	CO_2 inhalation
	C	0.0368	0.0592	14	30	520	Recovery
4	A	0.0491	0.0513	16	60	2560	Quiet breathing
	B	0.0399	0.0028	20	40	4000	CO_2 inhalation

Expt. 3. July 7, 1926. Brain weight 53.2 grams. Vertebral venous samples. Adrenalin 1:200,000 in perfusing blood throughout. Animal very anemic. Perfusion begun at 2:28.

A. 3:2:35. Oxygen by tracheal catheter throughout. Pump 88.23 cc. per minute. O_2(A)6.40 − (V)3.82 = 2.58 per cent or 2.276 cc. per minute. CO_2(V) 17.29 − (A) 13.14 = 4.15 per cent or 3.662 cc. per minute.

B. 3:6. CO_2 10 per cent, O_2 90 per cent on by tracheal catheter. 3:7. Pump 88.23 cc. per minute. O_2(A)6.36 − (V)4.02 = 2.34 per cent or 1.963 cc. per minute. CO_2(V)19.52 − (A)20.34 = *minus* 0.82 per cent or *minus* 0.723 cc. per minute.

C. 3:7:30. CO_2 off. O_2 on by trachea. 3:15:40. Pump 65.21 cc. per minute —reduced because of failing circulation. O_2(A)6.31 − (V)3.30 = 3.01 per cent or 1.963 cc. per minute. CO_2(V)19.45 − (A)14.45 = 5.00 per cent or 3.155 cc. per minute.

C. *Drugs.* The effects of morphine, atropine, caffeine, ether and ephedrine are shown in table 4.

In the cases of immediate respiratory failure following morphine or atropine, the effect on apparent cerebral oxygen consumption was a marked reduction, while the apparent CO_2 production showed an actual reversal—

that is, cerebral venous blood contained less CO_2 than arterial blood immediately after respiration had failed. It must be remembered that these were perfusion experiments, in which the blood supply of the brain was not permitted to adjust itself to changes in functional activity. In experiments on intact (non-perfused) animals no such reversed diffusion of CO_2 was found after respiration had been paralyzed by atropine or morphine.

In the cases of more gradual development of morphine depression, and in cases in which morphine failed to depress breathing; in the cases of caffeine, ether, and ephedrine—it is evident that apparent cerebral oxygen consumption follows closely the change in respiration.

The terminal injections of cyanide were made to determine the degree to which the other procedures had depressed the ability of the brain cells to utilize oxygen.

3. The reversal. In the preceding paper we have expressed the belief that the "Reversal" is the result of an interference with the fundamental (vegetative) functions of the cells of the central respiratory mechanism, produced by deficiency of oxygen within these cells. In support of this belief is the observation (table 1 of the preceding paper) that the apparent oxygen consumption of the brain is reduced when, following stimulation of the medulla during decreased blood-flow, respiration is depressed instead of stimulated. Further support is found in the results of attempts, in this and in two other experiments, to improve respiration through increasing cerebral blood-flow after the reversal had occurred. These results are shown in table 5. They indicate not only that the apparent ability of the brain to take oxygen from the blood was reduced as the result of the procedures employed to induce the reversal (prolonged reduction in cerebral blood-flow alone, or combined with electrical stimulation of the medulla), but that the ability to absorb oxygen was not restored by subsequent improvement of cerebral flow. The behavior of respiration followed fairly closely the curve of apparent oxygen consumption by the brain, for it improved most in the case of greatest improvement in oxygen absorption. The conclusion does not depend wholly on the fact that a smaller factor for blood-flow may dominate the calculation of oxygen absorption by the brain, for the percentile loss of oxygen by blood pumped through the brain was definitely less after the reversal than it was before, even though the pump output could not be increased to the preëxisting normal because the animal's circulation could not deliver enough blood for such output. And in the case in which respiration recovered this coefficient of cerebral oxygen utilization returned to its normal level, while in the other instances, in which there was no improvement of breathing, the coefficient did not approach normal. These facts are shown in the protocols of these experiments.

We regard these findings as evidence in support of the belief that de-

TABLE 4

Effect of drugs on apparent cerebral metabolism

NUMBER		CEREBRAL METABOLISM		RESPIRATION			CONDITIONS
		O_2	CO_2	Rate	Depth	M-V	
		cc. per gram per min.	*cc. per gram per min.*				
1	A	0.0677	0.1002	64	40	2560	Quiet breathing
	B	0.0558	−0.0393	0	0	0	After morphine (40 mgm.)
2	A	0.0259	0.0626			300	Respiration failing
	B	0.0664	0.0968			1000	After caffeine (40 mgm.)
	C	0.0064	−0.0197	0	0	0	After morphine (20 mgm.)
3	A	0.0788	0.1017	60	40	2400	Quiet breathing
	B	0.0821	0.0942	60	70	4200	After caffeine (15 mgm.)
	C	0.0141	−0.0032	0	0	0	After atropine (1 mgm.)
4	A	0.1017	0.1130	32	65	2080	Quiet breathing
	B	0.0718	0.0562	28	20	560	After morphine (10 mgm.)
	C	0.0331	0.0400	0	0	0	After morphine (total 70 mgm.)
5	A	0.0440	0.0252	12	15	180	Respiration failing
	B	0.0299	0.0861	0	0	0	After KCN (100 mgm.)
6	A	0.0472	0.0446	20	130	2600	Quiet breathing
	B	0.0383	0.0017	0	0	0	Medulla punctured
	C	0.0343	0.0379	0	0	0	After KCN (100 mgm.)
7	A	0.0693	0.1011	18	40	720	Quiet breathing
	B	0.0364	0.0627	0	0	0	Spontaneous periodic breathing
				20	40	800	
	C	0.0935	0.1586	25	60	1500	Caffeine (10 mgm.)
	D	0.0647	0.0471	0	0	0	Spontaneous failure of breathing
	E	0.0655	0.0559	0	0	0	After morphine (40 mgm.)
8	A	0.0613	0.0854	24	65	1520	Quiet breathing
	B	0.0602	0.0681	16	70	1120	After morphine (240 mgm.). Reflexes very active
	C	0.0241	0.1704	0	0	0	After NaCN (500 mgm.)
9	A	0.1492	0.1408	50	42	2100	Quiet breathing—no oxygen in trachea. Reflexes ++
	B	0.1864	0.1863	60	50	3000	Ether inhalation. Reflexes sluggish
	C	0.1309	0.1176	60	30	1800	Deep ether. Reflexes abolished
	D	0.1338	0.1518	60	40	2400	Recovery. Reflexes +++. Oxygen on
	E	0.0911	0.0594	30	10	300	Morphine (20 mgm.). Reflexes ++
	F	0.0844	0.0711	22	20	440	Caffeine (20 mgm.). Reflexes ++
	G	0.0755	0.0919	26	10	260	Morphine (140 mgm.). Reflexes ++
	H	0.0807	0.1318	50	15	750	Ephedrine (40 mgm.). Reflexes ++
	I	0.0571	0.1213	0	0	0	NaCN (500 mgm.). Reflexes 0

Expt. 2, table 4. June 10, 1926. Brain weight 22.8 grams. Vertebral venous samples. Oxygen by tracheal catheter throughout. No adrenalin. Perfusion begun at 1:10.

A. 2:2. Pump 28 cc. per minute. O_2(A)8.88 − (V)6.77 = 2.11 per cent or 0.591 cc. per minute. CO_2(V)25.11 − (A)23.41 = 1.70 per cent or 0.476 cc. per minute. Respiration very weak—failed during collection.

B. 2:6:30. Caffeine 40 mgm. by pump. Respiration began 20 seconds later. 2:10—Pump 28.6 cc. per minute. O_2(A)9.76 − (V)3.40 = 6.36 per cent or 1.516 cc. per minute. CO_2(V)29.48 − (A)21.76 = 7.72 per cent or 2.208 cc. per minute. Respiration fluctuated during collection, averaging about 1000 cc. per minute.

C. 2:16—Morphine sulphate—20 mgm. by pump. Respiration failed suddenly 2 minutes later. 2:22—Pump 28.3 cc. per minute. O_2(A)9.33 − (V)8.81 = 0.52 per cent or 0.147 cc. per minute. CO_2(V)28.65 − (A)30.24 = *minus* 1.58 per cent or *minus* 0.450 cc. per minute.

ficiency of oxygen in the respiratory center leads to a diminution in or loss of its ability to carry on its specific function because it depresses the fundamental (vegetative) functions of its cells: this is apparent in a prolonged reduction in the ability of the brain cells to take oxygen out of the blood under such circumstances.

4. Control procedures. The interpretation of our results regarding the apparent metabolism of the brain requires some assurance on two major points: First, as to whether this metabolism represented only that of the central nervous system; second, as to whether the changes in apparent metabolism were due to changes in functional activity of the central nervous system. Concerning the first of these we have the injections made after each experiment, to show that the venous blood was derived from the central nervous system. Since the jugular veins were never interfered with, there is no reason why blood from extracranial areas should be diverted to the cerebral sinuses, or venous return from the brain should be regarded as abnormal except as regards the right vertebral vein between collection of blood samples: this was always taken into consideration by allowing the collecting system to empty itself very thoroughly before blood samples were taken. We feel safe in concluding that the venous blood samples were derived wholly from the cerebro-spinal sinuses.

The other point—the relation of changes in apparent metabolism to changes in functional activity of the brain—is not so simply disposed of. It is proper to inquire, first, concerning the fluctuations in apparent metabolism to be encountered when no changes in functional activity of the brain are evident; and, second concerning the possibility of changes in activity of extracranial tissues playing a part in the change in apparent metabolism of the brain.

One successful experiment was made in which conditions were kept as constant as possible and blood samples were collected at 30 minute intervals. The results are shown in table 6. They show that the variation

TABLE 5

Apparent cerebral metabolism during the reversal

NUMBER		CEREBRAL METABOLISM*		CEREBRAL FLOW	RESPIRATION			CONDITIONS
		O_2	CO_2		Rate	Depth	M-V	
		cc. per gram per min.	*cc. per gram per min.*	*cc. per min.*				
1	A	0.0669	0.0501	53.57	20	60	1200	Quiet breathing—adequate flow
	B	0.0976	0.1004	0	28	70	1960	Acute cerebral anemia*
	C			57.69	0	0	0	Immediately after restarting pump
	D	0.0661	0.0614	55.69	24	60	1440	Eight minutes later
	E	0.0879	0.0536	56.60	40	50	2000	Electric stimulation of medulla—adequate flow
	F			56.60	24	50	1200	Two minutes later—adequate flow
	G	0.0347	0.0473	24.19	20	85	1700	Seven minutes after reduction of flow
	H			24.19	40	30	1200	Seven minutes later
	I	0.0280	0.0321	23.25	10	35	350	Electric stimulation of medulla—reduced flow
	J			23.25	5	10	50	Three minutes later
2	A	0.0762	0.1040	51.72	32	55	1760	Quiet breathing—adequate flow
	B	0.0350	0.0363	18.07	16	120	1920	Reduced flow—stimulant phase
	C	0.0343	0.0200	17.05	12	60	720	Reduced flow—reversal beginning
	D	0.0491	0.0513	36.81	16	160	2560	Flow improved 4 minutes before. Recovery
3	A	0.0752	0.0847	56.60	40	45	1800	Quiet breathing—adequate flow
	B	0.0380	0.0496	22.98	44	55	2420	Reduced flow—stimulant phase
	C	0.0325	0.0180	21.17	14	28	392	Same—reversal beginning
	D	0.0268	0.0151	23.07	16	8	128	Same—15 minutes later
	E	0.0361	0.0037	40.00	20	5	100	Flow improved 2 minutes before. No recovery

* In estimating metabolism during the period of acute anemia it was necessary to use the figure for pump output (57.69 per minute) just after the anemia was terminated. Vertebral venous blood also could not be collected until the pump was restarted, but the collection was begun simultaneously with the restarting. This blood therefore pictures the events of the anemia, and the apparent oxygen consumption is increased because the cerebral blood was more venous than before. Also, the hyperpnea of the anemia was replaced by the usual apnea upon restarting the pump, but since the hyperpnea obviously corresponded with the greater venosity of cerebral blood, it seems justifiable to tabulate them together.

Expt. 2. July 12, 1926. Brain weight 59.3 grams. Vertebral venous samples. Oxygen by tracheal catheter and adrenalin in perfusing blood throughout. Perfusion begun at 2:50.

A. 3:38—Pump 51.72 cc. per minute. O_2(A)15.92 − (V)7.18 = 8.74 per cent or 4.520 cc. per minute. CO_2(V)24.93 − (A)13.00 = 11.93 per cent or 6.170 cc. per minute. Respiration and reflexes very active.

B. 3:49—Pump slowed to 18.07 cc. per minute. 3:54 O_2(A)14.18 − (V)2.68 = 11.49 per cent or 2.076 cc. per minute. CO_2(V)22.31 − (A)10.38 = 11.93 per cent or 2.156 cc. per minute. Respiration increased, reflexes as active as before.

C. 3:59—Pump 17.05 cc. per minute. O_2(A)14.95 − (V)3.00 = 11.95 per cent or 2.037 cc. per minute. CO_2(V)21.64 − (A)9.96 = 11.68 per cent or 1.991 cc. per minute. Respiration failing, reflexes less active.

D. 4:0—Pump increased to 36.81 cc. per minute. O_2(A)14.82 − (V)6.91 = 7.91 per cent or 2.912 cc. per minute. CO_2(V)15.68 − (A)7.40 = 8.28 per cent or 3.048 cc. per minute. Respiration definitely improved, reflexes no more active. Complete recovery of respiration followed.

Expt. 3. July 13, 1926. Brain weight 66.1 grams. Vertebral blood samples. Oxygen by trachea and adrenalin by pump throughout. Perfusion begun at 2:19.

A. 2:40—Pump 56.60 cc. per minute. O_2(A)16.35 − (V)5.86 = 10.49 per cent or 5.937 cc. per minute. CO_2 (V) 29.07 − (A) 19.17 = 9.90 per cent or 5.603 cc. per minute.

B. 2:45—Pump slowed to 22.98 cc. per minute. 2:50—O_2(A)15.79 − (V)4.85 = 10.94 per cent or 2.514 cc. per minute. CO_2(V)26.34 − (A)13.55 = 12.79 per cent or 2.939 cc. per minute. Reversal began during collection of these samples.

C. 2:56—Pump 21.17 cc. per minute. O_2(A)15.23 − (V)5.08 = 10.15 per cent or 2.149 cc. per minute. CO_2(V)28.01 − (A)22.55 = 5.46 per cent or 1.156 cc. per minute. Respiration weak.

D. 3:1—Pump increased to 23.07 cc. per minute. 3:11—O_2(A)10.63 − (V)2.92 = 7.71 per cent or 1.779 cc. per minute. CO_2(V)22.04 − (A)17.78 = 4.26 per cent or 1.003 cc. per minute. Circulation failed during collection and was restored by injections of animal's own diluted blood previously withdrawn. Respiration very weak.

E. 3:20—Pump increased to 40 cc. per minute. 3:25—O_2(A)11.99 − (V)5.99 = 6.00 per cent or 2.400 cc. per minute. CO_2(V)23.67 − (A)23.07 = 0.60 per cent or 0.240 cc. per minute. Respiration not improved. Failed 5 minutes later.

of apparent cerebral oxygen consumption under constant conditions of cerebral blood-flow and respiration is small.

As to the possibility that changes in activity of extracranial areas (particularly the respiratory muscles) might lead to changes in the gas content of blood collected from the right vertebral vein or the torcular, the evidence, both indirect and direct, is against it. The injection of the venous collecting system uniformly showed no direct communication with extracranial areas, that might participate in respiratory efforts, though this type of evidence loses some of its value when one considers that the injections were made after death of the animal, when the patency of vessels and the mechanical effects of muscular movements were probably different from the state of affairs existing when blood samples were collected. As direct evidence we submit the following: electrical stimulation of the medulla uniformly increased apparent cerebral metabolism when spontaneous breathing was retained (table 2). Similar stimulation, using a current strong enough to cause violent tonic contractions of the muscles of the neck, forelimbs and chest, did not increase the apparent metabolism after respiration had failed (table 2, expt. 6 C).

We think that the strongest argument in favor of the belief that the gas metabolism of brain cells is regularly altered when respiratory activity is altered is found in the regularity with which the two changes coincide when various procedures are employed in different animals. The changes that follow drugs such as morphine and atropine—which are not known to act directly on extracranial tissues in such a way as to explain these results—and the reduction of apparent metabolism by cyanide, appear to us to be beyond the range of possible coincidence or of action on tissues outside the brain. While we do not claim any absolute accuracy for our figures for apparent metabolism, we believe that changes in this metabolism in the same direction as a result of the same procedures applied to different animals are as close to crucial evidence on this aspect of the problem as can be obtained with a system as complicated as the cerebral circulation of the breathing animal.

TABLE 6

Extent of spontaneous variation in apparent cerebral metabolism

NUMBER	CEREBRAL METABOLISM		RESPIRATION			CONDITIONS
	O_2	CO_2	Rate	Depth	M-V	
	cc. per gram per min.	*cc. per gram per min.*				
1	0.1342	0.1028	52	70	3640	Quiet breathing—time 3:25
2	0.1293	0.1227	40	90	3600	Same—time 4:05

The results of all our observations (excepting those made with cyanide) are shown in relation to the corresponding activity of respiration in figure 1. The points on these curves are the averages of the figures for apparent cerebral oxygen and CO_2 metabolism when respiration had failed, when breathing was less than 500 cc. per minute, when it was between 500 and 1000 cc. per minute, and so on. The appearance of these curves in relation to the activity of respiration is perhaps the strongest evidence we can submit on this aspect of the problem.

COMMENT. The results of these experiments have been, on the whole, more uniform and striking than we had any reason to expect. The curve of all our data (fig. 1) shows clearly the tendency of apparent cerebral metabolism to vary with activity of respiration, and various measures designed to alter respiration have all yielded similar results in different animals, with the single exception of CO_2 inhalation. These facts we interpret as evidence that the metabolic activity of the central nervous system of the anesthetized dog is of sufficient intensity to be considered a factor in the regulation of respiration.

We realize that this interpretation may be questioned. To assume that the "respiratory center" is capable of altering its metabolic activity to such an extent as to produce a gross change in gaseous metabolism of the entire brain would be to attribute an inconceivable grade of metabolism to a group of cells that is ordinarily regarded as a very small part of the total brain mass. But we do not regard it as possible that a measure which alters respiratory activity has acted solely upon the "respiratory center" of the medulla. It is an inevitable consequence that other cell groups concerned in vegetative functions should also be affected, and not im-

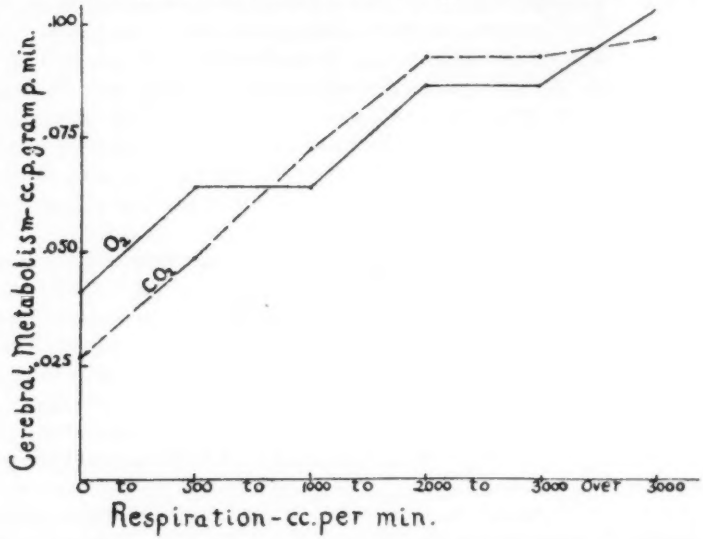

Fig. 1. Curve of apparent cerebral metabolism averaged from 11 estimations with no respiration; 9 with respiration under 500; 12 with respiration between 500 and 1000; 18 with respiration 1000 to 2000; 9 with respiration 2000 to 3000; 10 with respiration above 3000 cc. per minute.

probable that a considerable part of the brain should be involved in the action. This is particularly the case with drug effects, and it may also be true of all the other measures we have used, though probably to a lesser extent. If one regards the change in respiratory activity as an index of a change in functional activity of actively metabolizing parts of the central nervous system, it is not at all beyond the range of possibility that total apparent cerebral metabolism should vary constantly with respiratory activity. Another possibility is that the central respiratory mechanism constitutes a considerable part of the total brain mass. This is strongly suggested by the work of Lumsden (18), which led to the conclusion that

there are several "respiratory centers" at different levels of the brain stem above the medulla. The number of nerve cells, in motor cortex and other parts of the brain, which must be connected with the medullary "Respiratory Center," can only be guessed at.

It must be admitted that the changes in apparent cerebral metabolism may have coincided accidentally with the alterations in respiratory activity. The only answer we can furnish to this objection is the constancy with which the two changes coincided when various procedures were employed to alter respiratory activity in different animals, and the indirect evidence, presented in the preceding paper, that the respiratory responses to changes in cerebral blood-flow are such as to indicate an active metabolism in the central respiratory mechanism. A crucial experiment would involve isolation of this central mechanism and a study of the grade of its metabolism under conditions of altered respiratory activity. This is impossible by present methods, because the limits of the central respiratory mechanism are unknown, and even if they could be drawn accurately, the experimental procedures necessary to isolate it would preclude a convincing metabolism experiment.

There remains another objection to the desired interpretation of our results—namely, that the changes in apparent metabolism had nothing to do with changes in functional activity of any part of the central nervous system, but may have depended on redistribution of blood in the brain. If one could draw a picture of the tissues to which the perfused blood was distributed, and from which cerebral venous blood was withdrawn, in our experiments, it would probably show a number of small scattered areas with a high metabolic rate, surrounded by a huge mass of cells with a very low metabolism. These would represent the parts concerned in maintenance of respiration, circulation, etc., on the one hand, and the narcotized "higher centers" on the other. It is not only possible but highly probable that the distribution of blood among these parts should be subject to fluctuation from instant to instant: we have shown in the preceding paper that such vascular readjustments are possible. In fact, the only explanation we can offer for the reduction in apparent cerebral oxygen consumption of the brain during CO_2 inhalation is based upon such vascular readjustments. For CO_2 dilates cerebral vessels, and we have already shown that the response of cerebral vessels to acute anemia and to alkali is lost when cerebral functional activity has ceased. Consequently, one has good reason to suppose that the dilatation produced by CO_2 would be greater in the active parts of the brain than in the others. The result would be a relative increase in the amount of blood diverted to the most active tissues so that each unit of blood issuing from these parts of the brain may have lost less oxygen than before, though these active tissues may have taken out a greater total quantity of oxygen per minute

than before. This would entail a relative decrease in blood-flow through other parts of the brain, but since these are presumably in a state of depressed function the character of blood leaving them would not be altered much by a moderate decrease in their blood supply. It is possible, therefore, that the actual metabolic activity of the central respiratory mechanism was increased by CO_2, as it was by all other respiratory stimulant measures, in spite of the apparent decrease.

Obviously it would be inconsistent to evoke such vascular readjustments as an explanation of a discrepancy in our results, and to deny them a part in the results obtained by other measures than CO_2. It does not seem probable, however, that respiratory stimulant measures should uniformly decrease blood-flow through actively metabolising parts of the perfused tissue, and that respiratory depressant agents should uniformly increase it—the only vascular readjustment that could account for our changes in apparent cerebral metabolism. In fact our evidence is all to the contrary, for we have found that CO_2, acid, or oxygen lack dilates cerebral vessels, and such vascular changes as might occur in active parts of the brain would tend to minimize a change in apparent cerebral metabolism, just as they seem to have done in the case of increased CO_2 content of the blood.

An inspection of the tables given above, and of the protocols appended to this paper, will show that the figures for apparent CO_2 production are subject to much wider fluctuations than are those for apparent oxygen consumption. Our experience with the brain is in full accord in this respect with the results obtained by others upon leg muscle (22), (23), intestine (21), kidney (19) and heart (20), and we can only conclude, as have recent writers on the subject (23), that the CO_2 elimination of any tissue is influenced by unknown factors other than the CO_2 production at that instant, so that it is not as good a criterion of metabolic activity, at any instant, as oxygen absorption by the tissue. Our results with morphine, atropine, and cyanide show the oxygen absorption decreased by each, but while cyanide usually increased apparent CO_2 production, morphine and atropine, in acutely depressant dosage, decreased it to the point of an actual reversed diffusion—that is, cerebral venous blood actually contained less CO_2 than arterial blood in these cases. In one case a similar reversed diffusion of CO_2 was evident during CO_2 inhalation, in another following puncture of the medulla. We shall not attempt to explain these phenomena at present. The cyanide results, at least, are probably due to the use of enormously excessive concentrations. Yet because of the wide fluctuations in apparent CO_2 production of the brain, our data indicate that on the whole this figure shows a more striking correspondence with respiratory activity than is the case with the apparent oxygen consumption (fig. 1).

SUMMARY

1. The gaseous metabolism of the brains of dogs, perfused with their own blood, was estimated under conditions of altered respiratory activity.

2. This apparent metabolism varies widely in different animals, but tends to parallel the existing degree of respiratory activity.

3. When various measures are used to alter respiratory activity, apparent cerebral metabolism varies directly with respiratory activity with only one exception, namely, CO_2 inhalation.

4. The Reversal is shown to coincide with a prolonged reduction in the ability of the perfused brain to take oxygen from the blood, and recovery coincides with an improvement in this ability. The Reversal is therefore apparently due to a toxic effect of oxygen-lack and not to a simple narcotic effect.

5. The averages of all these results show that both apparent oxygen consumption and CO_2 production of the perfused brain vary directly with respiratory activity, the CO_2 metabolism being in rather closer agreement with respiration though subject to wider individual fluctuations than the oxygen metabolism.

6. It is suggested that the reduction in apparent cerebral oxygen consumption produced by CO_2 inhalation is due to dilatation of vessels supplying active parts of the brain.

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THE INFLUENCE OF CEREBRAL BLOOD-FLOW ON RESPIRATION

III. The Interplay of Factors Concerned in the Regulation of Respiration

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The most important conclusion to be drawn from the facts presented in the two preceding papers is this: the activity of the respiratory center depends on the concentration within its cells of stimulant material derived from metabolism. The intracellular concentration of this material is determined not only by the vigor of metabolism of the center but also by the rate of cerebral blood-flow: upon this last-named factor depends retention or escape of this material from the cells of the center. Increase or decrease in cerebral blood-flow causes respiration to change in the sense which would be anticipated if the circulatory change favored or impeded removal of stimulating material from the center.

There is therefore every reason to expect the response of the respiratory center to a change in rate of removal of its metabolic products (internal stimuli), resulting from a change in cerebral blood-flow, to be identical in all respects with its response to a change in chemical composition of the blood (external stimuli). This is not the case, for while the response to increase in the substance which is most representative of what we mean by stimulant material, namely CO_2, can be maintained indefinitely, that to change in cerebral blood-flow is transitory. As this difference necessitates consideration of factors which modify the response of the center to changes in its external and internal stimuli—a consideration that is the chief purpose of this paper—certain examples will now be presented.

Concerning the respiratory response to inhalation of CO_2, we have stated (1) that in the absence of anoxemia or circulatory depression CO_2 may be exhibited in concentration as high as 80 per cent and the result is pure stimulation.[1] These inhalations have been continued as long as

[1] In a recent experiment on a cat we have observed marked respiratory depression resulting from inhalation of 50 per cent CO_2 in oxygen. Blood pressure, at a normal level before, fell sharply early in the inhalation, then recovered, and the greatest respiratory depression coincided with the recovery of blood pressure. This is the first instance of respiratory depression by CO_2 alone that we have encountered, and since it followed recovery from a subnormal blood pressure the factor of oxygen lack in the center cannot be excluded.

15 minutes in six different animals with normal blood pressure, with a result uniformly like the example shown (1—fig. 11).

When cerebral blood-flow is markedly increased during inhalation of air or oxygen, respiration is first depressed, but if the increase in flow be maintained breathing gradually becomes normal. An example is shown in figure 1, cerebral flow being increased by continuous infusion of adrenalin so adjusted as to maintain blood-pressure at the same higher level. This shows also that an additional increase in flow again causes respiratory depression, while a return to the previous level causes respiratory stimulation. On discontinuing the infusion respiration becomes dyspneic at once, long before blood pressure has returned to normal. In this instance cerebral blood-flow was not measured, but we know from many other experiments that cerebral blood-flow is markedly increased whenever blood pressure is elevated by adrenalin.

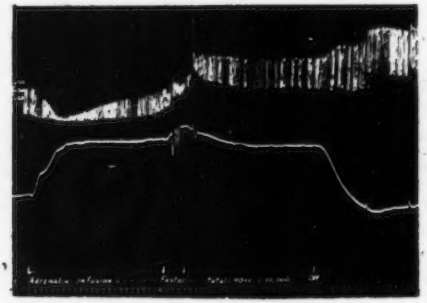

Fig. 1

When cerebral flow is decreased (fig. 2) the usual result is hyperpnea, but this does not persist even though cerebral flow continues at the lower level. Respiration either quiets down and approaches normal (fig. 2A), in which case a subsequent increase in cerebral flow depresses breathing, or it passes into the reversal stage (fig. 2B), when an increase in flow stimulates breathing.

Likewise in perfusion experiments (1—fig. 9) respiration is immediately affected by a change in cerebral blood-flow, but it tends as a rule to return to normal though the output of the pump be maintained at the altered level.

These results show that the agency which governs the respiratory response to alteration in cerebral blood-flow is the change in flow rather than the maintenance of a new level of flow. Evidently a change in cerebral flow introduces mechanisms which automatically compensate the influence of the change in flow upon the concentration of stimulant material within the center.

The most obvious of such mechanisms is a compensatory change in chemical composition of the blood resulting from the alteration in respiration. During the respiratory depression produced by adrenalin the CO_2 tension of the blood must rise, and its increasing influence upon the center might eventually nullify the increased discharge of stimuli resulting from the accelerated blood-flow through the center. The hyperpnea resulting from decreased cerebral flow must lower CO_2 tension in the blood, with corresponding effects on escape of metabolites from the center. The

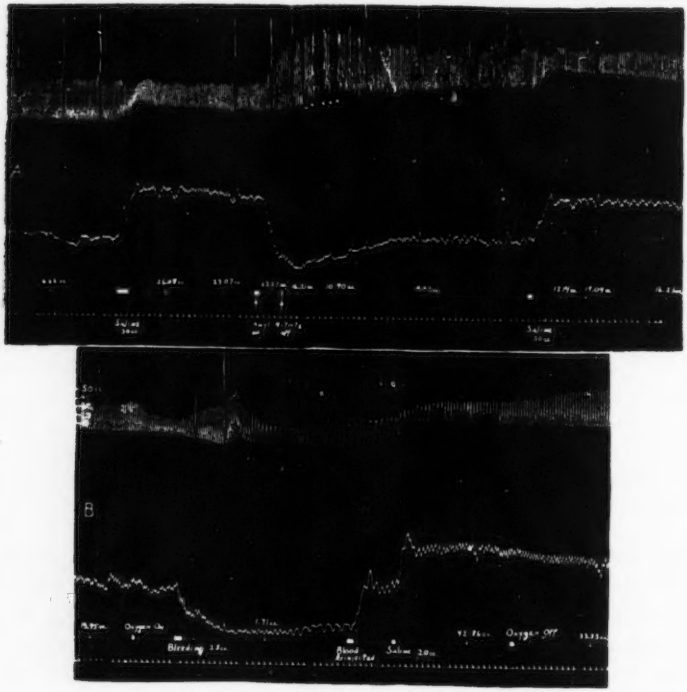

Fig. 2

vagus reflexes, described by Heymans and Heymans (3), are another possible factor in the escape of respiration from the effect of altered cerebral blood-flow. It is possible that the center can escape from these reflex influences, or that the reflexes are subject to "fatigue." There is no evidence that this is true. But there is evidence that neither blood-gas changes nor reflexes constitutes the chief factor in the transitory nature of the response to a sudden increase in cerebral blood-flow: immediate but transitory respiratory depression occurs when the cerebral vessels are

released, following brief occlusion, during CO_2 or nitrogen inhalation (1—fig. 8). Here the blood-gas content is such as to produce dyspnea when cerebral flow is normal, and the increase in cerebral flow coincides with a fall in aortic pressure, which should stimulate breathing reflexly (3), yet brief apnea occurs.

Evidently there is some other factor which antagonizes the influence of a change in cerebral blood-flow upon the respiratory center, or else our conclusion that change in cerebral flow acts through changing the concentration of stimulant material within the center is incorrect. Our experiments indicate that such a factor is found in the changes in cerebral vessels which follow changes in chemical composition of the blood or in total cerebral blood-flow. When the interplay of these vascular adjustments is studied it is evident that this is a factor of major importance in determin-

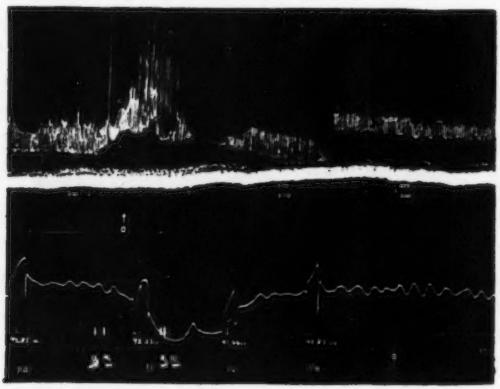

Fig. 3

ing accumulation or escape of stimulant material from the cells of the center, and therefore in the regulation of respiration.

I. The influence of changes in cerebral vessels. We have already shown (1) that the cerebral vessels are dilated by CO_2, acid, and oxygen-lack, and are constricted by excess oxygen or alkali. This fact, together with the demonstration that the activity of the respiratory center varies inversely with cerebral blood-flow, suggests that changes in the vessels supplying the respiratory center antagonize the influence of changes in chemical composition of the blood upon it. Our experiments show that respiratory responses coincide with changes in cerebral vessels which are in accordance with this inference.

For example (fig. 3) the substitution of pure oxygen for nitrogen in the inspired air leads to an apnea. Respiration soon returns and gradually

becomes normal. Coincidentally the cerebral vessels constrict, as is shown by rise of perfusion pressure. In such cases, the cerebral vessels having been dilated by anoxemia, the first completely oxygenated blood flows rapidly through the center. The subsequent recovery of respiration can be explained as being due to reduction of blood-flow through the center by constriction of its vessels. The stimulation of respiration produced by bicarbonate (4) (16) may also be explained by constriction of the blood vessels supplying the respiratory center, for we have shown that bicarbonate constricts cerebral vessels.

The lack of respiratory depression resulting from excess oxygen or alkali in the blood can thus be attributed to an antagonism between cerebral circulatory adjustments and the change in the blood, by virtue of which metabolites accumulate within the center and the external (blood) change is opposed. An analogous antagonism should also exist between an excess of CO_2 or other stimulant material in the blood and the cerebral vasodilatation produced by such agents, for the latter effect, by promoting interchange between the cells of the center and the blood, would minimize the accumulation of stimulant material produced within them. Unfortunately we cannot demonstrate this antagonism in the case of increased CO_2 or acid or decreased oxygen in the blood, for the cerebral vascular changes are not of vasomotor origin and cannot be prevented as long as the central nervous system continues active. It is impossible therefore to determine with any accuracy the part played in the respiratory response by the cerebral vascular change produced by an increase in external (blood) stimulant material. But it is possible to show that the dilatation of cerebral vessels produced by an accumulation of metabolites during acute cerebral anemia does diminish markedly the influence of external stimuli upon the center. Thus (1—fig. 7) blood which had maintained quiet breathing produced an apnea when it was readmitted to the cerebral vessels immediately after a period of acute anemia, and the return of respiration coincides with return of tone to the vessels. In these cases there could have been no change in the blood itself to account for the apnea.

Such experiments are therefore direct evidence that otherwise adequate chemical stimuli in the blood are rendered ineffective by a brief period of acute cerebral anemia. The simplest explanation is that dilatation of blood vessels supplying the respiratory center has facilitated discharge of stimulant material from within its cells, and this occurs as soon as blood is readmitted, though the blood reaching the center is identical with that which had previously been associated with normal breathing. Compensation depends upon a return of tone to the vessels supplying the center, permitting metabolites again to accumulate within it.

The inferences from such experiments are directly applicable to the respiratory responses to increased stimulant material within the blood.

For there is good reason to believe that the respiratory and cerebral vascular events of acute cerebral anemia are due to accumulated products of nerve cell metabolism, and that the products of nerve cell metabolism are similar to those of other tissues (2). If cerebral vasodilatation antagonizes the influence of constant blood stimuli upon the center in the case of acute cerebral anemia, it is a reasonable assumption that similar antagonism must exist between an increase in external stimuli and the cerebral vasodilatation produced by such increase.

The assumption that changes in caliber of vessels supplying the respiratory center antagonize the influence of changes in blood stimuli upon it has so far been based upon the coincidence of such vascular adjustments with changes in the stimuli. To establish the validity of this assumption it is necessary to show that a change in caliber of the vessels supplying the center is able to modify the activity of the center when all else remains constant. We have obtained evidence in certain perfusion experiments

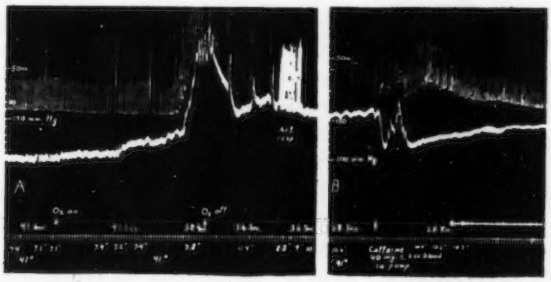

Fig. 4

that this is true. Reference has been made (1) to the occurrence of intense constriction (spasm) of the perfused vessels in some of these preparations, and the relaxing effect of vasodilator drugs. Examples are shown in figures 4 and 5. In one case (fig. 4) the spasm followed oxygen inhalation and was allowed to continue until the Reversal occurred: caffeine promptly dilated the perfused vessels and respiration improved at once—the usual respiratory response to an increase in cerebral blood-flow after the Reversal. In the other (fig. 5A) the spasm followed an increase in pump output, which caused first a depression of breathing, followed by dyspnea: caffeine was given during the dyspneic stage of the respiratory response before the Reversal had occurred, and it produced the immediate respiratory depression that is typical of a sudden increase in blood-flow through the normal center. Figure 5B is taken from a subsequent part of this experiment, when respiration had failed following a period of cerebral anemia: it shows the return of spontaneous breathing coinciding with a return of tone to the perfused vessels.

Such experiments prove, we think, that constriction (spontaneous) of the vessels supplying the respiratory center is able to produce stimulation followed by depression of breathing, and that dilatation of these vessels (by drugs) can elicit the effects of an increase in blood supply of the center. The respiratory phenomena are identical with those resulting from a decrease or increase in total cerebral blood-flow. As a result, we conclude that changes in the vessels of the respiratory center are able to modify the response of the center to changes in the chemical composition or total flow of blood reaching the center, and that the assumption of an antagonism between blood stimuli and cerebral vascular changes is a valid one. If the activity of the center depends on the concentration of stimulant material within its cells, a change in external stimuli is constantly opposed by a compensatory change in internal stimuli, through the medium of adjustments in the vessels supplying the center. In the cases of oxygen inhalation and bicarbonate injection the vascular change appears to dominate the respiratory response. In the case of CO_2 inhalation, acid injection and anoxemia one can only infer that the antagonism is operative in minimizing the respiratory response.

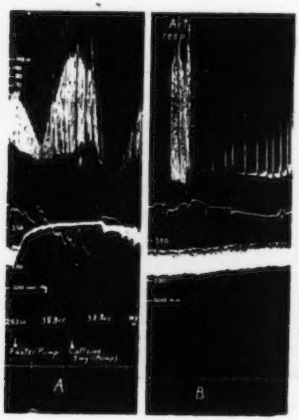

Fig. 5

In the cases of an increase in total cerebral blood-flow, constriction of the cerebral vessels can be demonstrated in some cases, at least (fig. 5), and it may be assumed to occur as a typical response, so that the change in total flow is opposed. Likewise when total cerebral flow is decreased, the cerebral vessels dilate, so that stimulant material can be more readily discharged from the center and the respiratory response decreases (fig. 2A); if total flow is reduced to a point at which metabolites cannot be effectively removed from the center in spite of cerebral vasodilatation the result is not a continuation of the stimulant response, but the Reversal (fig. 2B). We have not found any intermediate point between these two extremes at which respiration can be continuously stimulated by a given reduction in total cerebral blood-flow—it either returns to normal or the Reversal occurs.

There is therefore no reason to believe that the respiratory center is affected differently by external agents than by internal ones, or products of its own metabolism, in spite of the transitory nature of the response to the changes in cerebral blood-flow. For in the latter cases the internal stimulant is opposed not only by changes in the blood vessels supplying the center, but also by compensatory changes in external (blood) stimuli

In the former the change in the blood is continued, and is opposed only by changes in internal stimuli, through the vascular adjustments referred to above.

The part played by this antagonism between external stimuli and cerebral vascular changes in the normal regulation of breathing is a matter of conjecture at present except in the case of oxygen or bicarbonate excess, where the relation is evident. It is not improbable that because of these vascular changes the respiratory center is less sensitive to changes in the blood than it would be otherwise, so that a marked response is reserved for changes in the blood which might prove detrimental to the body as a whole. It may be that these vascular changes can be made more perfect by repeated exposure to the exciting agents, and that the phenomena of "training" are dependent upon training of the vessels supplying the respiratory

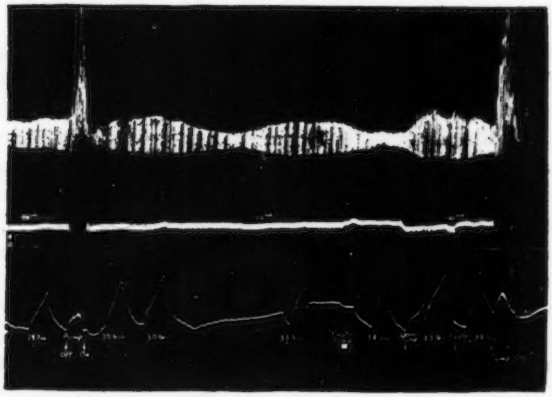

Fig. 6

center; "second wind" may be due to a sudden improvement in the blood supply of the respiratory center by dilatation of its vessels. It is not impossible also that the vascular changes play a part in preventing exhaustion of the oxygen supply of the center when it is violently stimulated, as by vigorous muscular exercise: this entails a greater demand for oxygen by the entire body, including the respiratory center (2), and the cerebral vascular change is a mechanism admirably suited to insure the supply to the center, failing which the Reversal would occur.

II. THE SIGNIFICANCE OF THE VASOMOTOR RESPONSE. In the preceding section we have called attention to an antagonism between internal and external respiratory stimulant agents, exerted through a change in the vessels supplying the respiratory center. The fact that the vasomotor center is stimulated by the same agents which stimulate respiration, and

that a vasomotor response entails an increase in cerebral blood-flow, leads to the conclusion that a similar antagonism exists between the vasomotor and respiratory responses—that the vasomotor response promotes escape of metabolites from the respiratory center, thereby minimizing the response of the latter and increasing its adaptability to excess of external stimulant material. In addition, we have found evidence that the vasomotor response is particularly concerned with two phases of the regulation of breathing.

The first of these has to do with stabilizing the respiratory response to external or internal stimuli, and with restoring respiration to normal when the stimulant is withdrawn. An example of the instability of the respiratory center when a vasomotor response is prevented is shown in figure 6. In this—a perfusion experiment—acute cerebral anemia was produced by stopping the pump. Respiration was stimulated, and on restoration of cerebral blood-flow it was depressed, recovering gradually, as usual. For no evident reason, there followed a period of respiratory depression; then one of spontaneous recovery. At this point the pump was accelerated and respiration was immediately depressed. Later the pump was slowed and breathing increased. The respiratory effects of these artificial changes in cerebral flow are analogous to the changes occurring spontaneously while flow was constant after the anemic period.

The suggested explanation is based on changes in blood-gas content resulting from the respiratory reactions. The hyperpnea produced by the period of acute cerebral anemia reduced the CO_2 tension of arterial blood. When, after the interval necessitated by the capacity of the pump system, this blood reached the brain, respiration was depressed. During this period CO_2 again accumulated in the blood, and when this blood reached the brain respiration was again stimulated. This cycle might have gone on much longer had it not been interrupted by an artificial increase in cerebral blood-flow, but the immediate stabilizing effect of this increase suggests that a vasomotor response would have had similar effects.

We believe that the vasomotor response to a change in blood stimuli tends to restore breathing to normal: that in the absence of such vasomotor responses an irregularity of respiration once induced would go on until it could be checked by readjustments in cerebral vessels and in the acid-base balance of the blood. As the vasomotor response is more rapid in its effects than these other mechanisms, it seems to play a large part in abolishing respiratory irregularities.

The other manifestation of the vasomotor response in the regulation of respiration has to do with the Reversal. We have shown (2) that respiration may fail if the center is not supplied with the increased oxygen demanded by its cells when they are stimulated. Ordinarily this is taken

care of by a vasomotor response and dilatation of cerebral vessels. But in poisoning by narcotic drugs, particularly with morphine, the vasomotor center is depressed and blood pressure low (5) (6). The cerebral vessels must be dilated by the cerebral anemia resulting from the low blood pressure. The only mechanism left to furnish an increased supply of oxygen to the center is the vasomotor response to the stimulant, and if this fails the Reversal is likely to occur. We have found that whenever a stimulant (CO_2, caffeine, etc.) raises blood pressure it stimulates breathing, but if blood pressure fails to rise, or particularly when it falls, the Reversal is likely to occur. We regard CO_2 inhalation as unsafe when it fails to raise blood pressure in conditions of subnormal cerebral blood-flow.

There is therefore reason to believe that depression of the vasomotor center limits the ability of the respiratory center to respond to stimuli, and that absence of a vasomotor response is the exciting cause of the Reversal following respiratory stimulants such as CO_2, caffeine, etc.

III. The part played by changes in the metabolic activity of the center. The extent to which internal stimuli (metabolites) of the center may be concerned in the regulation of breathing can be inferred from the fact that respiration is depressed by a sudden increase in cerebral blood-flow though the chemical stimuli of the blood be unaltered or markedly increased (1—figs. 7 and 8). Unless other unknown factors are concerned this can only mean that the concentration of stimulant material within the center reaches a level above that of the blood when cerebral blood-flow is reduced, even though the blood level be such as to produce dyspnea under normal conditions of cerebral flow. The immediate respiratory response to a change in cerebral blood-flow indicates that the equilibrium between metabolites of the center and the blood supplying it is a delicate one. All these results point to a high grade of metabolism in the center.

If it is granted that the center has a metabolism it is only reasonable to suppose that the intensity of this metabolism should vary from time to time. Such changes in internal stimuli might well be responsible for the changes in blood stimuli which are attributed to changes in "irritability" or "threshold" of the center. Also, it is possible that afferent nervous influences alter the metabolic activity of the center. In favor of this possibility is our demonstration (2) that electrical stimulation of the central end of a vagus nerve or of the medulla itself alters the apparent metabolism of the brain in the direction of the change in respiration. Viewed in this light, the nervous regulation of respiration operates through changes in rate of production of chemical stimuli within the center and is therefore part of the chemical regulation. This interpretation rests only upon the coincidence of altered cerebral metabolism with nervous alterations in respiratory activity. It is equally probable that the metabolism

is altered because the nervous influence has altered the functional activity of the central respiratory mechanism—that the change in metabolism was the result rather than the cause of the change in respiration—but the other possibility seems to us to be worthy of consideration.

The action of morphine, caffeine, atropine, ether, and cyanide upon cerebral metabolism (2) is so marked as to leave little doubt that these drugs are able to alter the rate at which stimulant material is produced within the respiratory center. The coincidence of altered metabolism and respiratory change is more striking here than in the case of nerve stimulation. It is possible therefore that the change in respiration produced by these drugs is due to the change in metabolism of the center, though here also it is impossible at present to distinguish cause from effect.

Changes in metabolism of the center may also be concerned in the escape of respiration from the influence of a change in chemical composition of the blood or of a change in total cerebral blood-flow—an escape which has been attributed in the preceding sections to compensatory changes in the blood supply of the center. There is at present no evidence that the production of internal stimuli is altered to compensate for changes in external stimuli, but there is evidence that the accumulation of internal stimuli is so altered, through the cerebral vascular changes already described.

IV. Periodic respiration. That the respiratory center is affected by changes in its blood supply we regard as established. It follows that periodic changes in cerebral blood-flow, no matter how produced, will produce periodic respiration. Our experiments have shown that there are three possible types of periodic breathing due to primary circulatory changes. These are as follows:

1. Apnea at the highest level of blood pressure, respiratory activity at its maximum at the lowest level of pressure (with a light lag in each case). This is the response of the normal respiratory center, and the condition is commonly encountered in the early stages of experimental narcotic poisoning, the circulatory changes being evidently due to rhythmic activity of the vasomotor center. When it occurs mean blood pressure is little if at all below normal and respiration is not seriously depressed.

2. Apnea at the low level of blood pressure, respiratory activity at its highest level (again with a slight lag)—the Reversal type of response. It is seen most strikingly in cases of high intracranial tension, where there is almost complete anemia of the center until a vasomotor response is able to establish enough circulation through it to restore respiration. A similar relation is sometimes encountered as a nearly terminal event in experimental narcotic poisoning, but here the circulatory changes appear to be more the result than the cause of the respiratory rhythm. This type of periodic breathing means serious depression of the center, and we regard

it as due primarily to oxygen-lack within it. Mean blood pressure is typically well below normal in these cases except those which are due to increased intracranial tension: in these it may be above normal and it cannot fall very far without permanent respiratory failure.

3. Periodic constriction and dilatation of the blood vessels supplying the respiratory center—the probable cause of periodic breathing without changes in blood pressure and without any evident cause, instances of which are occasionally encountered in animal experiments. More commonly the vessels of the vasomotor center appear to participate in the periodic change, and the picture is that of an intermittent asphyxial circulatory and respiratory response: dyspnea coincides with a rise in blood pressure, apnea with a fall.

Examples of the first of these types are given by Barbour (7), and Eyster (8) shows instances of the periodic breathing of increased intracranial tension. The third is largely hypothetical, but we think we have sufficient evidence upon which to base the claim that primary changes in cerebral vessels can produce marked respiratory effects; we do not know however if such changes occur in man, or even in animals which are not subjected to cerebral perfusion, with its attendant abnormalities.

The importance of this grouping of types of periodic breathing lies in the fact that the underlying mechanisms differ and the significance of the three conditions is not the same. As long as the apneic period coincides with or follows closely the peak of the blood pressure curve, the respiratory center is not seriously depressed, and the periodicity indicates rather an instability of the vasomotor center than dangerous depression of respiration. But when apnea occurs after blood pressure has fallen considerably serious oxygen-lack within the center is indicated, and prevention of permanent respiratory failure depends on improving the supply of oxygen to the brain: oxygen inhalation, circulatory stimulation, and lowering of intracranial tension if it is high, are the measures to be applied. When respiration is periodic in the presence of normal blood pressure and intracranial tension, without any simultaneous periodic changes in blood pressure, periodicity may be due to spontaneous changes in the vessels supplying the respiratory center; or if, blood pressure and intracranial tension being normal, dyspnea coincides with a rise in blood pressure, apnea with a fall, simultaneous changes involving the vessels of vasomotor and respiratory center are suggested; in either case respiration should be made regular by vasodilator drugs, but these would be fatal in the second type. Differentiation between the second and third types rests on the condition of blood pressure and cerebro-spinal tension: in either respiratory activity would coincide with a rise in blood pressure, apnea with a fall, but in the second mean blood pressure is low, or if high, intracranial tension is increased markedly; in the third mean blood pressure and cerebrospinal tension are normal or nearly so.

We do not claim that all forms of periodic breathing are caused by primary changes in cerebral blood-flow, and this classification is intended to include only those which are so caused. Our information is not complete enough to attempt an evaluation of all the factors which may be concerned in the production of periodic breathing. In many cases the changes in blood pressure may be the result rather than the cause of the respiratory reaction. But we believe that the statements made above, concerning the significance of the relation of the apneic periods to the circulatory changes, are applicable to any type of periodic breathing, no matter how produced. Apnea outlasting the higher level of blood pressure means a center in good condition, apnea throughout the lower level means serious oxygen-lack in the center, and should be treated accordingly.

V. The regulation of respiration. The major conclusion to be drawn from our experiments may be stated as follows: the chief function of the entire respiratory mechanism is the maintenance within the cells of the center of a constant concentration of stimulant material. The factors involved in this maintenance are those enumerated in our tentative hypothesis, namely: First, changes in the arterial blood, produced chiefly by changes in alveolar ventilation, to a lesser extent by the other mechanisms involved in the regulation of neutrality in the body (9); second, changes in rate of metabolism of the cells of the center, possibly accounting for changes in "irritability" of the center and for nervous influences upon it; third, changes in rate of removal of metabolites from the center, chiefly through changes in the vessels supplying it, also through vasomotor responses, both sets of influences antagonizing chemical changes in the blood. The end result is a delicate balance between external and internal stimuli, a balance which can be disturbed either from without (through changes in the blood) or from within (through changes in rate of production or removal of metabolites) with the same result, namely, a change in concentration of stimulant material within the center and a corresponding respiratory response.

We do not propose to amplify the term *chemical stimulant material.* As far as the general conception is concerned it makes no difference whether one calls it CO_2 tension, oxygen tension, acid-base balance, or total acidity. But it should be noted that once oxygen-lack comes into play, the respiratory response may be modified by the development of the Reversal. Since CO_2 excess does not seem to be so limited as a respiratory hormone it seems fair to regard it as a more important single agent than oxygen-lack, and there is abundant evidence in the work of Haldane and co-workers (10) that the center is much more sensitive to CO_2 excess than to oxygen-lack in the blood. We have no original data concerning acid-base balance or total acidity as compared with CO_2 or anoxemia. It seems to us that attempts to evaluate the relative importance of ions or equilibria among

ions as respiratory stimulant agents cannot lead to unequivocal conclusions until more is known about the intervention of cerebral vascular adjustments. Thus, we are reasonably certain that increased oxygen or bicarbonate in the blood stimulates breathing because it constricts the blood vessels of the center.[2] We do not know whether increased CO_2 causes more or less dilatation of these vessels than oxygen-lack or increased acidity—if less, some of the apparently specific effects of CO_2 could be accounted for. It may also be that the serious derangement which we call the Reversal begins much earlier with oxygen-lack or acid excess than we suspect at present. For the present we think it best to regard the chemical stimulant material concerned in respiratory control as the product of metabolism of living cells, including those of the center itself, without attempting to single out any particular metabolite as a specific stimulant.

At the present stage of this investigation we arrange the agencies concerned in normal respiratory regulation as follows, in the order of their apparent importance: First, the CO_2 tension or acidity of arterial blood; second, adjustments in the vessels supplying the center, antagonizing the change in the blood and preventing the Reversal; third, the vasomotor response, also antagonizing the change in the blood and preventing the Reversal; fourth, changes in metabolism of the center—this may be more important than we can forsee, but it is put last because accurate information is lacking.

In addition to these, two other factors are probably concerned in regulating the concentration of stimulant material within the cells of the center. These are changes in permeability and in buffer content of those cells. Both have been mentioned by Gesell (11). Their evaluation is obscured at present by the intervention of cerebral vascular adjustments and their place in the above grouping cannot be assigned even tentatively until more information is available. The interplay of reflexes with internal or external chemical stimuli has not been studied. Apparently reflexes operate in the same direction as chemical changes in the blood (3) in most cases and do not antagonize them.

The conception of respiratory control that has been developed in the course of these experiments makes possible a reasonable explanation of some drug effects that were formerly inexplicable (5) (6). It is essen-

[2] According to this reasoning, carbonate should be more stimulant to breathing than bicarbonate because it produces more intense cerebral vasoconstriction (1). Yet carbonate produces only respiratory depression (16). Evidently the result of the antagonism between a change in blood pH and constriction of vessels of the respiratory center cannot be predicted, but must be determined by actual experiment in every case.

tially the same as the theory of Gesell (11), and we regard our results as evidence to be added to that obtained in Gesell's laboratory in favor of the tenability of this theory, though our experiments were made for a different purpose and by different methods. This conception of the regulation of respiration differs from Haldane's theory (10), not in the nature of the regulating material, but in its localization within the center instead of in the arterial blood or alveolar air, and this involves the additional factors of metabolism and blood supply of the center in the regulation. Within recent years attention has been drawn to the rôle of metabolism of the center by Winterstein (24) and by Gesell (12), and the part played by changes in the vessels of the center has been alluded to by Haldane (25) and Barcroft (26). Since the realization of the importance of these factors seems to be the most recent trend of thought concerning respiratory control, it is proper to note that this conception was developed and clearly stated many years ago by Rosenthal (27), whose theory of the regulation of breathing took into consideration the metabolism of the center and the effects of changes in cerebral blood flow, as well as the gas content of arterial blood. But Rosenthal differed from most subsequent workers in that he regarded the oxygen content of the blood as the chief agent in the normal regulation of breathing, and it is for this reason that his entire conception has been discarded. Thus, according to Haldane (10), the weak spot in Rosenthal's theory is the absence of apnea when pure oxygen is breathed. However, in work done in Haldane's laboratory (28) the conclusion was reached that oxygen may actually stimulate breathing because it constricts the vessels supplying the respiratory center, thus leading to an increase in CO_2 tension within the cells. The absence of apnea during oxygen inhalation would therefore point to a participation of the metabolism of the center in its own regulation—a conception which we believe to be valid—but this is in accordance with Rosenthal's theory, not opposed to it. Granted that the work of Haldane and others (10) has shown that Rosenthal was wrong in regarding blood oxygen as the chief factor in normal respiratory control, this objection applies only to part of his theory: if one substitutes *chemical stimulant material* for Rosenthal's *oxygen content*, his theory becomes identical with the conception developed in the preceding pages.

The demonstrations (10) of the exquisite sensitivity of the respiratory center to changes in blood CO_2 tension or acidity led to concentration of attention upon the hormone regulation of breathing, in spite of the fact that, in the words of Gesell (11), "the exceptions continue to be the rule." Only recently have definite attempts been made to develop a theory which should explain the respiratory events of oxygen-want, of hemorrhage, of acute cerebral anemia. The result has been a reversion to the older idea of Rosenthal—namely, that the metabolism and blood supply of the center are concerned in the regulation of breathing.

The first of these of which we are aware was an essay written by Pearce in 1919 (29). It contained the suggestion that the CO_2 pressure of blood leaving the center determined the CO_2 pressure within the center, so that a change in the blood supply of the center would affect respiration—a suggestion upon which we based the tentative hypothesis used in designing our experiments. Soon afterward Winterstein (24) stated that the hyperpnea of anoxemia is a "centrogenous" one—that is, dependent upon acids produced within the center—in contrast with that of CO_2 or acid excess, which is "hematogenous." In the course of the investigations made by Pike and coworkers on the phenomena of acute cerebral anemia was a series of experiments (30) which led to the conclusion that acute anemia causes dyspnea because there is not enough blood to remove CO_2 from the center as fast as it is formed. The latest development has been the statement of the theory of Gesell (12), and the appearance of publications from his laboratory in support of it (12 to 23).

None of these workers has attempted to evaluate the interplay of cerebral vascular changes in the regulation of respiration. This aspect of the problem has been approached recently by Haldane (28), and Barcroft (26) points out that an improvement of the blood supply of the center is the mechanism most likely to be effective in adjusting the respiratory system to sustained low oxygen tensions. The assumptions made by them involve also the third factor in Rosenthal's theory—namely that the metabolism of the center plays a part in the regulation of respiration—though Haldane has recorded the conviction (10) that the center is affected more by changes in the blood than by products of its own metabolism. This is based on the absence of respiratory stimulation during the reduction in cerebral blood-flow of fainting, though he presents no evidence either that cerebral flow is of necessity reduced in fainting, or that depression of the medullary centers is not a primary factor in producing the fainting. We regard it as definitely established that respiration is stimulated by a reduction in cerebral blood-flow short of the Reversal. And the implications of Haldane's recent development of the idea that the center may be stimulated by constriction of its vessels produced by increased oxygen (28), and of Barcroft's suggestion that it may be depressed by dilatation produced by low oxygen pressure (26), are definitely in harmony with the conception of the regulation of respiration which was first clearly stated by Rosenthal, subsequently alluded to by Pearce and Winterstein, and recently redeveloped by Gesell.

SUMMARY AND CONCLUSIONS

1. The chief function of the entire respiratory mechanism is the maintenance within the center of a constant concentration of stimulant material. Factors known to be concerned in this maintenance are: changes in arterial

blood, through changes in pulmonary ventilation, acid excretion by kidneys, etc.; changes in metabolic activity of the center, possibly concerned in changes in irritability of the center and in the reflex regulation of breathing; changes in blood supply of the center, through local vascular and general vasomotor responses, which influence removal of metabolites from the center. Respiratory activity at any instant depends upon a delicate equilibrium among these factors, and can be altered by changing any one of them.

2. A primary change in accumulation of metabolites within the center (internal stimuli) leads to a compensatory and antagonistic change in chemical composition of the blood (external stimuli), through the respiratory response. Similarly a primary change in external stimuli leads to a compensatory and antagonistic change in internal stimuli, through changes in the blood vessels supplying the center and through a vasomotor response. Consequently the concentration of stimulant material within the center tends toward normal, and the respiratory response is minimized: in the case of excess oxygen or bicarbonate in the blood the expected response is reversed by constriction of vessels supplying the center.

3. The nature of the stimulant material responsible for the regulation of respiration cannot be determined until more definite information is available concerning the part played by changes in the vessels supplying the center. The material is derived from metabolism of cells, including those of the center, and it is probable that CO_2 is more important to the normal regulation than oxygen-lack because the latter tends to modify the activity of the center by producing the Reversal.

4. Changes in cerebral blood-flow coinciding with changes in respiratory activity are probably concerned in dampening the respiratory response, in restoring breathing to normal without irregularities after it is stimulated, and in preventing the Reversal during violent chemical stimulation.

5. Periodic breathing of three distinct types can be produced by primary changes in blood supply of the center. The significance of these types differs widely, and it is probable that similar reasoning can be applied to all types of periodic breathing, no matter how produced.

The writer wishes to record his deep appreciation of many helpful suggestions, during the course of these experiments, and of active assistance in preparation of these manuscripts, which it was his privilege to receive from Prof. A. N. Richards. The assistance of Dr. A. E. Livingston in arranging apparatus for measurement of cerebral blood-flow is gratefully acknowledged.

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